Methotrexate and Dipyridamole Combination Chemotherapy Based upon Inhibition of Nucleoside Salvage in Humans

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ABSTRACT

We have carried out a clinical trial in 23 patients to determine whether dipyridamole modulates the clinical effect of methotrexate. This trial was based upon in vitro studies which indicate that dipyridamole potentiates the cytotoxic action of methotrexate through inhibition of thymidine salvage. Methotrexate was given as a bolus injection 24 h after initiation of a high dose dipyridamole infusion. The trial was designed so that methotrexate was escalated in individuals until toxicity occurred and then the methotrexate dose resulting in toxicity was repeated without dipyridamole. During the course of this study the methotrexate dose was escalated from 10 to 130 mg/m². While individual patient tolerance varied, moderate to severe myelosuppression and/or mucositis occurred frequently in patients receiving the combination with methotrexate doses ≥60 mg/m². Ten of 10 patients who experienced moderate or severe toxicity with the combination had significantly less toxicity when treated with methotrexate alone. Dipyridamole did not increase toxicity by an alteration in methotrexate elimination. The potentiation of methotrexate by dipyridamole in these patients suggests that physiological thymidine levels are sufficient to perturb the clinical effects of methotrexate and that thymidine salvage may represent a mechanism for clinical resistance to methotrexate. These results also suggest that a high dose dipyridamole regimen can be used as a pharmacological approach to test the role of nucleoside membrane flux on the clinical action of other standard chemotherapeutic drugs. Phase II studies testing the clinical efficacy of this combination should use a methotrexate dose of 60 mg/m² with a provision for methotrexate dose escalation based upon individual patient tolerance.

INTRODUCTION

The salvage of thymidine from extracellular pools is a mechanism by which cells may circumvent methotrexate toxicity. Evidence for the role of thymidine salvage in the reduction of methotrexate toxicity comes from studies in cultured cells (1-4) and animal models (5), as well as in patients treated with methotrexate and pharmacological doses of thymidine (6-9). In these models thymidine and purine nucleosides are taken up by cells from the extracellular pool by facilitated membrane transport and metabolized via the salvage pathway to restore the thymidylate and purine nucleotide pools depleted by methotrexate. As a consequence, thymidine and a purine source can reverse the cytotoxic action of methotrexate. Despite such preclinical models and clinical studies, thymidine salvage has not been considered a prominent mechanism for clinical resistance to methotrexate. It has been argued that the circulating thymidine pool is not sufficient to reverse methotrexate toxicity through action of the salvage mechanism (1). Results from in vitro studies have called this assumption into question. We found that thymidine uptake was stimulated in colon cancer cells following exposure to methotrexate and that a low thymidine concentration similar to levels found in human plasma was sufficient to restore TTP pools and reverse methotrexate toxicity (2). Howell et al. (3) found that physiological thymidine concentrations and hypoxanthine were sufficient to modulate methotrexate toxicity to human bone marrow granulocyte macrophage colony forming units. In this trial we evaluate the clinical significance of thymidine salvage in modulating methotrexate toxicity through use of dipyridamole as a pharmacological approach to block thymidine salvage in patients treated with methotrexate.

Dipyridamole inhibits nucleoside salvage by blocking the membrane uptake of nucleosides (10-15). Because dipyridamole is highly bound by plasma proteins, a high total plasma level must be achieved to establish the free dipyridamole plasma levels required to block nucleoside uptake. We have recently reported that it is possible to achieve pharmacologically active free drug levels by means of a high dose infusion of dipyridamole and that these dipyridamole levels are tolerated satisfactorily in patients with solid tumors (16, 17). In the present study we combined this high dose dipyridamole regimen with methotrexate in a phase I trial designed to determine if nucleoside salvage modulates methotrexate effect.

MATERIALS AND METHODS

Patient Selection

Individuals with advanced malignancy for whom no standard effective therapy was available, who gave informed consent according to institutional and Food and Drug Administration guidelines, and who had adequate bone marrow (WBC ≥4,000/mm³, platelet count >100,000/mm³), renal (serum creatinine ≤1.5 mg/dl and creatinine clearance ≥60 ml/min), and hepatic (aspartate aminotransferase <2× normal, alkaline phosphatase <2× normal, bilirubin ≤1.0 mg/dl) functions were eligible. Patients who had a performance status ≥2 on the ECOG scale; who had symptomatic coronary artery disease, central nervous system metastases, or a coagulation disorder; or who were taking either dipyridamole or theophylline were not eligible for the study.

Patients entering the trial had the following characteristics. All patients had advanced solid tumors and the majority had either colorectal, head and neck, or lung cancers; the median age was 60 (range, 38-76); 22 had a performance status of 0 or 1 (ECOG grade 18); and 19 had received prior treatment with chemotherapy (11), radiation (2), or both (6).

Drug Administration and Methotrexate Dose Escalation

Dipyridamole was administered as a 72-h continuous i.v. infusion via a central venous catheter at a dose of 23 mg/kg/72 h as described

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previously (16). The dipyridamole dose remained constant during the course of this trial.

Methotrexate was administered as a single i.v. bolus injection 24 h after the initiation of the dipyridamole infusion. The dose of methotrexate was escalated from a starting dose of 10–130 mg/m² during the course of this phase I trial in the following levels: level 1, 10 mg/m²; level 2, 20 mg/m²; level 3, 30 mg/m²; level 4, 45 mg/m²; level 5, 60 mg/m²; level 6, 80 mg/m²; level 7, 100 mg/m²; level 8, 120 mg/m²; and level 9, 130 mg/m². A minimum of 3 patients were treated at each dose level; and if no grade 3 or worse toxicity occurred, the next 3 patients were entered at a methotrexate dose level higher. Treatments were repeated every 3 or 4 weeks provided all toxicity had resolved. The methotrexate dose was escalated one level for each treatment course in individual patients provided that no grade 2 or worse toxicity occurred during the previous course. If grade 2 or worse toxicity occurred, then following resolution of toxicity the same methotrexate dose was administered without dipyridamole.

The maximum tolerable dose of methotrexate was defined as that dose level at which grade 2 mucosal or grade 3 nonmucosal toxicity (other than the nausea, vomiting, or headache associated with dipyridamole infusion) occurs in greater than one-third of the patients treated.

During the course of the trial hematological parameters and serum chemistries including hepatic, renal, and clotting functions were monitored weekly.

Frequency and Duration of Treatment

In patients with stable or responding disease and in whom all toxicity from a prior treatment had resolved were treated every 3 or 4 weeks. Patients continued to receive the combination until progressive disease or intolerable toxicity occurred.

Drug Formulation

Dipyridamole was supplied by Boehringer Ingelheim, Ridgefield, CT, in 2-ml sterile ampuls containing 10 mg of dipyridamole. The calculated 12-h dose was administered in 1 liter of normal saline. Methotrexate was prepared in the standard fashion and administered as an i.v. bolus at a peripheral site.

Analytical Techniques

Methotrexate Plasma Levels. Methotrexate plasma levels were determined for each course at the following intervals after bolus administration: 0, 15, and 60 min; and 3, 6, 9, 12, 20, 24, 30, and 48 h. Methotrexate was assayed by fluorescence polarization immunoassay procedure using the Abbott TDx analyzer (19).

Dipyridamole Assay. Dipyridamole total and free plasma levels were determined in all patients treated with dipyridamole prior to the infusion and at 24, 48, and 72 h. The high performance liquid chromatography assay for dipyridamole in plasma followed previously published methods (16).

Statistical Methods. The differences in nadir WBC in 9 patients when treated with methotrexate alone or when they received both methotrexate and dipyridamole were analyzed by paired t test and by a randomized block design with covariates, implemented by the SAS PROC GLM (20). In the randomized block design with covariates analysis the WBC on the first day of each treatment course and the methotrexate dose were controlled for as covariates with the treatment factor having three levels: the first course of dipyridamole and methotrexate; the second course of dipyridamole and methotrexate; and methotrexate alone. The Tukey’s studentized range test was used to rank the three levels of treatment. The paired t test was used to analyze the differences between the mean methotrexate clearance in patients treated with methotrexate alone or when given in combination with dipyridamole.

RESULTS

Methotrexate Dose Escalation Experience. A total of 23 patients entered the study and 70 treatment courses were administered. Myelosuppression was the dose limiting toxicity; however, as illustrated in Fig. 1, the myelosuppressive effects of the combination varied greatly among individual patients. For example grade 2 myelotoxicity occurred with 20 mg/m² methotrexate in patient C, while similar myelosuppression occurred at 80 and 130 mg/m² in patients L and G, respectively (Fig. 1). Prior treatment exposure, age, or differences in methotrexate clearance did not account for the variability.

Sixty mg/m² appears to be a safe starting dose for methotrexate when combined with this regimen of dipyridamole. One of 12 patients treated with methotrexate doses <60 mg/m² experienced myelotoxicity. Myelotoxicity was experienced in 7 of 16 patients treated with 60 mg/m². Individual patients tolerated higher methotrexate doses than 60 mg/m²; however, all patients were treated at the 60 mg/m² dose before escalation to a higher dose (Fig. 1). The maximum methotrexate dose administered in combination with dipyridamole in this trial was 130 mg/m². Median time to WBC nadir was 1 week and counts returned to baseline values by 2 weeks.

Thrombocytopenia occurred less frequently than leukopenia with the methotrexate and dipyridamole combination. Three of 6 patients which developed leukopenia after 60 mg/m² methotrexate had grade 3 thrombocytopenia. Platelet nadir was 1 week and counts returned to baseline by 2 weeks.

In addition to the hematological toxicities observed, 3 patients developed severe mucositis and 8 experienced an asymptomatic transient rise in serum aspartate aminotransferase following the combination with methotrexate doses of 60 mg/m² or higher. The median aspartate aminotransferase value was 102 units (range, 68–209 units). Bilirubin and alkaline phosphatase values did not rise. Moderate (grade 2, ECOG scale) or worse headache and fatigue were experienced by 12 of the 23 patients treated with the combination and 6 complained of moderate (grade 2, ECOG scale) nausea or vomiting.

One patient developed a profound myelosuppression and severe mucositis during the second course of dipyridamole and 60 mg/m² methotrexate. This was complicated by a fatal bacterial sepsis. The extreme toxicity in this patient was explained by an altered methotrexate clearance and the case is discussed further under “Methotrexate Pharmacokinetics.” All other toxicities during the trial resolved without sequelae.

Clinical Response. There was one partial response among the 23 patients entered. This response occurred in a hepatic metastasis from a gastroesophageal adenocarcinoma. The response
was documented by computer assisted tomographic scan and persisted for 2 months before progression. Twenty-one patients have expired with disease progression and 2 remain alive with disease.

Modulation of Methotrexate Toxicity by Dipyridamole. This trial was designed to determine if a high dose dipyridamole regimen could potentiate the clinical effect of methotrexate. In the trial we used the toxicity experience to assess the potentiation of methotrexate by dipyridamole. Patients who developed grade 2 or worse toxicity were treated with methotrexate alone at the dose which resulted in toxicity when given with dipyridamole. During the course of this trial 10 of the 10 patients who experienced grade 2 or worse myelotoxicity were treated with methotrexate alone. The individual WBC nadirs following the combination and methotrexate alone for the 9 patients in whom grade 2 or worse leukopenia occurred are shown in Fig. 2. In 6 patients a second course of the combination was administered under identical conditions. In these 9 patients the mean nadir WBC following treatment with methotrexate alone (4.6; n = 10) differed significantly from the mean nadir following both methotrexate and dipyridamole (1.9; n = 10) (P = 0.0008, paired t test). When the dose of methotrexate and the WBC on the first day of treatment cycle were controlled for as covariates, the mean nadir WBC with methotrexate alone also differed significantly from the nadir following the combination (95% confidence by Tukey’s studentized range test). The mean nadir WBC following a second course of the combination treatment (3.1; n = 6) was also significantly different from methotrexate alone but did not differ from mean nadir with the first combination treatment (95% confidence by Tukey’s studentized range test).

Three patients developed grade 3 thrombocytopenia with methotrexate and dipyridamole. One of the three had only grade 1 leukopenia. In all three cases methotrexate alone did not cause significant platelet count suppression. Three patients with grade 2 or 3 myelotoxicity also developed severe mucositis following the combination. In all three no mucositis occurred following methotrexate alone.

The transient elevation in aspartate aminotransferase transaminases which occurred in 8 of 23 patients receiving the combination was not experienced in 4 patients when treated with methotrexate alone. The remaining 4 did not receive methotrexate alone. While the data with methotrexate alone are limited, they suggest that dipyridamole also potentiated the transient hepatic toxicity seen in this trial.

No constitutional symptoms, headache, or gastrointestinal complaints were experienced in patients treated with methotrexate alone. These are all symptoms associated with the high dose dipyridamole regimen used in this trial.

Methotrexate Pharmacokinetics. We considered the possibility that dipyridamole potentiated methotrexate toxicity by altering methotrexate elimination. To evaluate this possibility parameters of methotrexate elimination were determined for each treatment course. As shown in Fig. 3, area under the concentration curve of methotrexate when administered with dipyridamole was directly proportional to dose, P < 0.001 (regression analysis). The methotrexate total body clearance (CL\textsubscript{TB} for all courses was 3.5 ± 0.10 liters/h/m\textsuperscript{2}. There was no difference in the CL\textsubscript{TB} for courses administered with dipyridamole, 3.6 ± 0.3 (SE) liters/h/m\textsuperscript{2} (n = 11), versus all courses with methotrexate alone, 3.1 ± 0.3 liters/h/m\textsuperscript{2}, P = 0.1 (paired t test). The methotrexate clearances for individual courses given with and without dipyridamole are shown (Fig. 4). If the CL\textsubscript{TB} for those courses with 60 mg/m\textsuperscript{2} are analyzed separately, there was no difference in CL\textsubscript{TB} when methotrexate was administered...
with dipyridamole, 3.5 ± 0.18 liters/h/m² (range, 2.0–4.9; n = 20), or without dipyridamole, 3.4 ± 0.56 liters/h/m² (range, 1.8–5.6; n = 6). Therefore the potentiation of methotrexate toxicity was not a result of an alteration in methotrexate elimination.

As discussed above a patient treated at the 60-mg/m² methotrexate dose developed myelosuppression which was complicated by a fatal bacterial sepsis. This event was unexpected because it occurred during the third treatment course in a patient who had experienced only moderate toxicity at the same dose of the combination and no toxicity when treated with methotrexate alone at 60 mg/m². The untoward toxicity experienced during the third course was explained by a dramatic decrease in methotrexate clearance from 2.7 liters/h/m² during the first course given with dipyridamole to 0.78 liters/h/m² with the course complicated by sepsis. The delayed clearance during the latter course resulted in exposure to a prolonged plasma level of methotrexate about 1 μM. We cannot exclude the possibility that dipyridamole or the combination altered methotrexate clearance; however, the first course did not demonstrate a dipyridamole effect on clearance and no other patient treated in this trial experienced a significant decrease in methotrexate clearance that was not explained by an associated change in renal function. Renal insufficiency which can lead to altered methotrexate clearance, coadministration of drugs which could alter methotrexate clearance, third space fluid accumulation, and an error in dose were all excluded as explanations for the delayed methotrexate clearance in this patient. An analysis of the methotrexate clearance during the first and third courses of patients receiving multiple courses of the combination showed no significant change (P = 0.34, n = 11). This experience suggests that if a cumulative change in methotrexate clearance occurs as a consequence of this combination such a result is uncommon. Following this event, we began to monitor the methotrexate plasma level at 24 h with the intent to administer leucovorin if a level above 0.5 μM was found. No subsequent patient required leucovorin rescue.

Dipyridamole Plasma Levels. The mean steady state dipyridamole levels during the infusion of 23 mg/kg/2 h were total dipyridamole, 5.5 ± 0.26 μM, and free dipyridamole, 20 nM ± 1.4. The percentage of free dipyridamole was 0.39 ± 0.17%. Fig. 5 shows the mean total (Fig. 5A) and free dipyridamole (Fig. 5B) values at 24, 48, and 72 h. Note that near steady state levels are achieved by 24 h and sustained through the completion of the 72-h infusion.

**Discussion**

The design of the study permitted a clear demonstration that dipyridamole potentiates methotrexate toxicity. While the results from this trial do not directly prove that this potentiation resulted from an inhibition of nucleoside salvage by dipyridamole, there are several observations which support this hypothesis. They include the results from preclinical models which demonstrate that salvage of low levels of thymidine is sufficient to replete TTP pools in cells exposed to methotrexate (2), the observation that dipyridamole at concentrations established in this clinical trial potentiates methotrexate through a block of thymidine uptake (10, 12), the observation in this phase I trial that dipyridamole did not alter methotrexate toxicity through changes in the clearance of methotrexate, and the fact that this regimen of dipyridamole did not cause hematological toxicity in a previous phase I trial (16). It is possible that we have overlooked an alternative mechanism to explain the modulation. For example, in a murine sarcoma model dipyridamole potentiates methotrexate cytotoxicity through inhibiting the efflux of methotrexate from the cell (21). The mechanism, however, required very high levels of dipyridamole, 10 μM free dipyridamole, which were 500-fold greater than the levels achieved in this trial, and it is unlikely that it explains the current findings.

The results of this trial are significantly different from recently reported phase I and phase II studies combining methotrexate with a p.o. regimen of dipyridamole (22–24). In these previous studies there was no evidence that dipyridamole modulated the effect of methotrexate. The dipyridamole serum levels achieved in these trials appear to be lower than those levels necessary to inhibit nucleoside salvage. Other clinical studies utilizing a p.o. dipyridamole regimen have documented peak levels of total dipyridamole which range from <1 to 3 μM and steady state levels below 1 μM (25-32) are achieved with p.o. doses. Therefore a key difference in this trial and those in which a p.o. dipyridamole regimen is used is that plasma concentrations achieved with the p.o. formulation are approximately 10-fold lower than the levels achieved in this trial. These are levels well below that level predicted by in vitro studies to be necessary to block thymidine uptake. An additional problem in the interpretation of the combination using a p.o. formulation is the erratic bioavailability of the p.o. formulation (26).

In contrast, the high dose infusion regimen used in the present trial results in a reproducible free dipyridamole plasma level which is in the range that in vitro studies would predict to be required for blockage of thymidine uptake. Furthermore in the high dose regimen this level is sustained 48 h after methotrexate exposure. Achievement of a steady state level may be a very significant factor, since the pharmacological effect of dipyridamole on nucleoside salvage is rapidly reversible. If alternative routes of dipyridamole delivery are to be developed for use in dipyridamole based combinations, these regimens should produce plasma concentrations in the range achieved with the i.v. infusion regimen used in this trial.
It is important to emphasize that in this trial only a modulation of toxicity has been demonstrated. Since the dose of methotrexate in the combination is lower than the methotrexate dose when given alone, it is possible that an equitoxic regimen of methotrexate alone will have identical clinical activity as the combination which requires lower dosage of methotrexate. Alternatively the data of Weber (12) strongly suggest that many cancer cells have a greater reliance on nucleoside salvage than normal tissues and that as a consequence the combination should have greater selectivity for the cancer cell. Ultimately the question of specificity can be answered only by phase III studies and we plan such a trial for advanced squamous cell cancer of the head and neck. This trial, however, suggests that physiological levels of thymidine do modulate methotrexate toxicity and it provides evidence that nucleoside salvage should be considered as a physiological resistance mechanism.

On the basis of this trial we can make the following recommendation for future methotrexate and dipyridamole studies. Dipyridamole, 23 mg/kg/72 h, is tolerated as a continuous infusion administered via a central venous catheter. A reasonable starting dose of methotrexate when combined with this dipyridamole dose is 60 mg/m². Individual patient tolerance will vary and in order to optimize anticancer effect we recommend increasing the methotrexate dose at subsequent courses if no grade 3 toxicity occurs. If our phase I trial experience is representative, one-half of the patients treated at the 60-mg/m² dose will tolerate higher methotrexate dosages. Because of the one experience where methotrexate clearance was dramatically delayed when administered with dipyridamole, we recommend that a methotrexate level be monitored 24 h after administration of methotrexate. If the 24-h methotrexate level is >0.5 μM, leucovorin rescue should be given.

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REFERENCES


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