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Phase II Evaluation and Plasma Pharmacokinetics of High-Dose Intravenous 6-Thioguanine in Patients with Colorectal Carcinoma*

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Summary. *A phase II study of intermittent high-dose 6-thioguanine (6-TG) was undertaken in 19 patients with metastatic colorectal carcinoma. Fourteen patients had received prior myelosuppressive therapy. 6-TG was administered as a single dose by IV bolus over 15–30 min, with retreatment every 3 weeks. The starting dose was 700 mg/m^2 in ten patients, 900 mg/m^2 in one patient, 1,000 mg/m^2 in four patients, and 1,200 mg/m^2 in two patients. Two patients received reduced doses (350 mg/m^2) because of liver dysfunction. There was no regression of measurable disease after treatment with 6-TG in this study. Eight patients achieved stabilization of previously progressive disease for periods of 10–32 weeks. Toxicities were nausea and vomiting (19 patients), mucositis (3 patients), reversible renal dysfunction with creatinine > 2 mg/dl (4 patients), nasal congestion (3 patients), diarrhea (1 patient), and skin blistering at the infusion site (1 patient). Seven patients had white blood count nadirs below 3,000/μl (the lowest nadir was 900/μl). Only one patient had a platelet count nadir below 100,000/μl. There were no infections or hemorrhage. 6-TG, as administered in this study, has no antitumor activity against colorectal carcinoma. Concentrations of 6-TG and metabolites were assessed in the plasma of six patients by a reversed-phase HPLC system. 6-TG and metabolites were extracted from human plasma at 50%–100% efficiency by cold 2 N perchloric acid (1 : 1). Neutralized extracts were chromatographed on a μ-Bondapak C_{18} column by two separate isocratic conditions. 6-TG, 6-thiouric acid, 6-thioguanosine, and 6-thioxanthine were analyzed with 0.01 M Na acetate, pH 3.5/10% methanol as the mobile phase and were detected at 340 nm. 6-Methyl TG and three unknown metabolites were eluted with Na acetate/25% methanol and were detected at 310 nm. External standard calibration was used for quantitation. The 6-TG detection limit was 0.8 nmol/ml. In six patients who received 1–1.2 g 6-TG/m^2 IV, 6-TG achieved peak plasma concentrations of 61–118 nmol/ml (95.6 ± 23.0, mean ± SD). Plasma 6-TG concentrations decayed bi-exponentially, with initial $t_{1/2}$ of 3 h and terminal $t_{1/2}$ of 5.9 h. 6-Thiouric acid, 6-methyl TG, 6-thioguanosine, 6-thioxanthine, and three major unidentified metabolites were also observed in plasma. The three unknowns were extracted with ethyl acetate from alkalinized pooled plasma extracts and were purified by HPLC.*

Introduction

Metastatic adenocarcinoma of the colon or rectum has a poor response rate to all antineoplastic chemotherapeutic agents. No single agent or combination of agents has produced a significant prolongation of survival in patients with this tumor [10]. Therefore, phase II evaluations of new drugs against colorectal carcinoma are important investigative efforts. 6-Thioguanine (6-TG), a purine antimetabolite, has been used principally in the treatment of acute nonlymphocytic leukemia, but information about the activity of 6-TG against human nonhematologic neoplasms is limited [4, 5]. In a study utilizing 6-TG at a daily dose of 1 mg/kg (30–35 mg/m^2), the Eastern Cooperative Oncology Group (ECOG) reported objective responses in four of 54 patients with refractory metastatic colon carcinoma [6]. In a subsequent phase I study by the Southeastern Cancer Study Group (SECG), 6-TG, at IV doses of 400–500 mg/m^2, was tolerated by patients, the major toxicity being myelosuppression [2]. From the expansion of this SECG phase I study, Presant et al. indicated that

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5/21 patients with metastatic colon or rectal carcinoma had objective responses to IV 6-TG given at doses up to 800 mg/m^2 [9]. Moreover, these large IV doses produced tolerable myelosuppression [9], the principal toxic effect of this drug [2, 5]. On the other hand, these large IV doses of 6-TG were associated with a high frequency of transient elevations in BUN, a toxicity not previously attributed to 6-TG [4, 5].

In that these studies of intermittent high-dose IV 6-TG suggested enhanced response with tolerable myelosuppression, it seemed appropriate to expand these early observations. We therefore conducted a phase II trial and studied the plasma pharmacokinetics of 6-TG in patients with colorectal carcinoma.

Materials and Methods

Patients with histologically confirmed metastatic colorectal adenocarcinoma who were admitted to the Baltimore Cancer Research Center between January 1980 and January 1981 were eligible for this study. Patients were required to have measurable disease, normal renal function (serum creatinine $\leq$ 2 mg/dl), a white blood count (WBC) > 3,500/μl, a platelet count > 100,000/μl, and a life expectancy of at least 2 months. In addition, eligible patients were required to be at least 6 weeks beyond their most recent surgical procedure, radiation therapy, or chemotherapy (8 weeks for nitrosoureas or mitomycin C). Informed consent was obtained from all patients prior to entrance to the study.

Standard criteria were used to evaluate an objective response [8]. A complete response was defined as the complete disappearance of all clinically evident disease for at least 30 days. A partial response was defined as a > 50% reduction of perpendicular dimensions of the largest diameter in all measurable lesions for at least 30 days. Stabilization was defined as < 50% reduction of measurable lesions or the lack of 25% progression of measurable disease for a minimum of 60 days.

6-TG was supplied by the National Cancer Institute (Bethesda, MD, USA). When clinically formulated 6-TG was reconstituted with sterile water, the pH of the 6-TG solution was approximately 11.5 and the drug irritated the patients' veins. Therefore, preparation of the drug was modified to lower the pH to approximately 9.5 without precipitating 6-TG from solution. We added 5 ml 0.154 *M* NaCl and 0.5 mg sodium bicarbonate to each 75-mg vial. Then an equal volume of 0.154 *M* NaCl was added to the above mixture. 6-TG was administered as a 30-min infusion every 3 weeks. The initial IV dose was 700 mg/m^2, although two patients with liver metastases and bilirubins > 3 were started at 350 mg/m^2. After a patient's first course of 6-TG, the dosage of each subsequent course for that patient was excalated by an additional 100 mg/m^2 if the WBC nadir during the preceding course remained above 1,500/μl and if the platelet count nadir remained above 100,000/μl. However, if the patient's WBC nadir during the preceding course was < 1,500/μl or the platelet count nadir was < 100,000/μl the dosage of the subsequent course was reduced by 200 mg/m^2. Also, if by day 21 the WBC count was below 2,900/μl and/or the platelet count was < 100,000/μl, then the subsequent 6-TG therapy was delayed for 1 week. Due to the lack of severe myelosuppression in the first 12 patients studied, the starting dose of 6-TG was escalated in subsequent patients. One patient received a starting dose of 900 mg/m^2, four patients received a starting dose of 1,000 mg/m^2, and two patients received a starting dose of 1,200 mg/m^2.

Pharmacokinetic studies of 6-TG and metabolites were performed in six patients who received 700–1,200 mg/m^2. At standardized times after injection of 6-TG, blood samples were collected in heparinized tubes and plasma was separated by centrifugation and stored frozen at −20° C until assayed. 6-TG and metabolites were extracted from plasma with perchloric acid and were assayed by high-performance liquid chromatography (HPLC) as previously described [1]. Plasma (1 ml) was mixed with 1 ml iced 2.0 *N* perchloric acid and was centrifuged at 4° C at 48,000 *g* for 20 min. Of the resulting supernatant solution, 1 ml was adjusted to approximately pH 11 with 4.0 *N* KOH. The alkalinized solution was stored at 4° C for 36–48 h to allow complete precipitation of $KClO_4$. Prior to injection onto the HPLC, the supernatant's pH was readjusted to approximately 3.0 with 1 *N* HCl. The HPLC system consisted of a Spectra-Physics (Santa Clara, CA, USA) Model 3500B high-performance liquid chromatograph fitted with a 3.9 mm × 30 cm μ-Bondapak C_{18} column (Waters Associates, Milford, Mass, USA) and utilized two separate isocratic conditions. 6-TG, 6-thioxanthine, 6-thiouric acid, and 6-thioguanosine were separated with a mobile phase of 10% methanol in 0.01 *M* sodium acetate, pH 3.5, pumped at 2 ml/min and were detected at 340 nm. 6-Methylthioguanine and three unidentified metabolites were separated with a mobile phase of 25% methanol in 0.01 *M* sodium acetate, pH 3.5, pumped at 2 ml/min, and were detected at 310 nm. 6-TG and metabolite standards were run with each set of plasma samples, and external standard calibration was used for quantitation.

Results

Nineteen patients with histologically proven metastatic adenocarcinoma of the colon or rectum were treated with 57 IV courses of 6-TG. All patients were evaluable for toxicity and response. The characteristics of the patient population according to age, sex, disease involvement, prior therapy and performance status are listed in Table 1.

No patient had a therapeutic response from 6-TG as given in this study. However, ten patients with previously progressive disease achieved stabilizations lasting from 10–32 weeks.

All 19 patients experienced some nausea but four patients experienced severe vomiting lasting more than 48 h. No patient required IV fluid administration for dehydration. Three patients developed mucositis without any complicating infections, one patient experienced diarrhea lasting several days, and one patient had infusion site skin blistering after extravasation of drug. It is of interest that four patients complained of nasal congestion and rhinorrhea, beginning within 24 h of administration of 6-TG and lasting for 3–5 days. In four patients, serum creatinine rose to 2 mg/dl or higher within 1 week of administration of 6-TG. One patient had a dramatic elevation of serum creatinine from 0.7 mg/dl to 3.6 mg/dl, but upon completion of treatment his serum creatinine returned to baseline. Another patient had a serum creatinine elevation from 1.8 mg/dl to 3.2 mg/dl but this was difficult to interpret since there was

Table 1. Patient characteristics

No. of patients entered	19
No. of patients evaluable	19
Age (years) Median	59
Range	37–70
Sex: Male	13
Female	6
Performance status	
Karnofsky ≥ 70%	10
< 70%	9
Prior therapy	
Chemotherapy alone	11
Chemotherapy + radiotherapy	1
Radiotherapy alone	2
None	5
Sites of involvement	
Liver only	6
Liver + local	4
Liver + lung	2
Liver + bone + local	2
Local only	2
Liver + bone	1
Local + lung + liver	1
Lung + local	1

also evidence of hydronephrosis on a pretreatment IV pyelogram.

The major toxic side-effect was leukopenia. Among the 16 patients who received 700–1,100 mg 6-TG/m^2, six patients (17/44 courses) had WBC count nadirs of 2,000–3,000 μl. One of the four patients who received 1,200 mg/m^2 (1/7 courses) had a WBC count nadir of 900/μl. One patient (1/57 courses) who received 800 mg/m^2 had a platelet count nadir of 67,000/μl. Seven of ten patients who began therapy at 700 mg/m^2 received sequentially higher doses. The one patient who started at a dose of 900 mg/m^2 had the dose escalated to 1,000 mg/m^2 for the second treatment, and one of four patients started at a dose of 1,000 mg/m^2 had the dose escalated to 1,100 mg/m^2. Two patients had dose reductions because of toxic effects: one patient, who received 1,200 mg/m^2, developed an elevated creatinine (3.6 mg/dl) and WBC nadir of 900/μl and the other patient, who received 1,000 mg/m^2, developed mucositis. There were no episodes of bleeding or infection and there were no drug-related deaths.

6-TG and several of its metabolites were detected in the plasma of patients (Fig. 1). Concentrations of

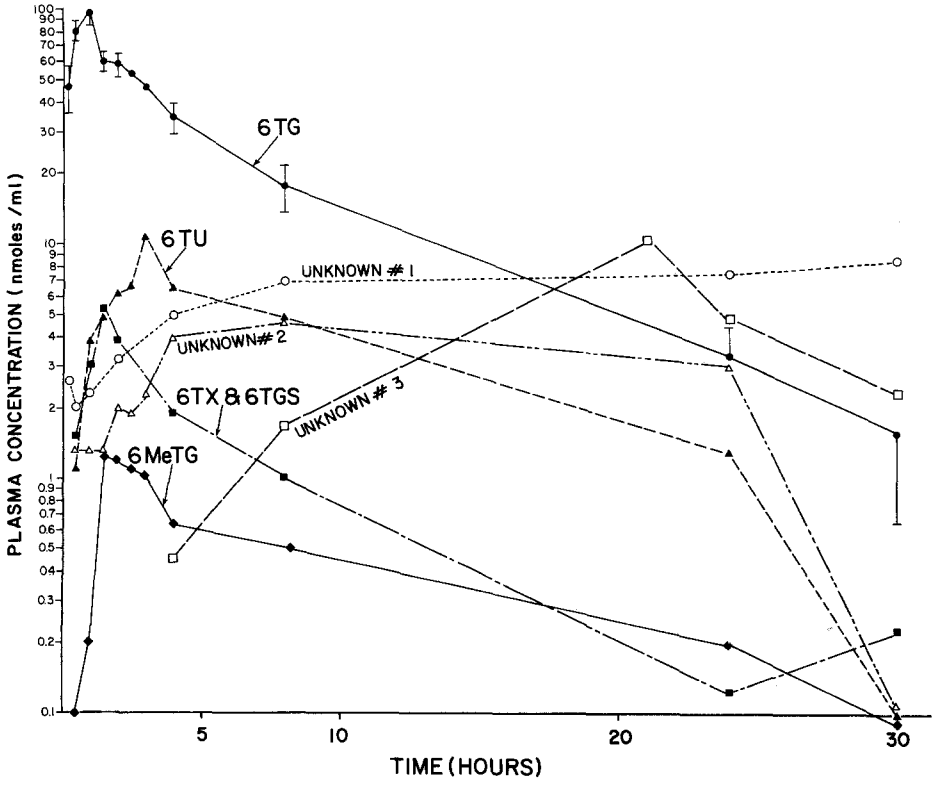

Fig. 1. Concentrations of 6-TG and metabolites in the plasma of patients treated with 800–1,200 mg 6-TG/m^2 IV. 6TG (6-thioguanine), 6TU (6-thiouric acid), 6TX (6 thioxanthine), 6TGS (6-thioguanosine), and three unknown metabolites were extracted from plasma with perchloric acid and were analyzed by reverse-phase HPLC as described in *Materials and Methods*. *Points* depict the means ± SE of determination from six patients

6-TG rose during the course of the infusion, peaking at 61–118 nmole/ml. After completion of the infusion, concentrations of 6-TG declined in bi-exponential fashion, the initial decline having a $t_{1/2}$ of 3.0 h and the slower terminal decline having a $t_{1/2}$ of 5.9 h. Several known metabolites of 6-TG were also observed in plasma (Fig. 1). Concentrations of both 6-thiouric acid and 6-methyl TG peaked somewhat later than did those of 6-TG, and then declined. At no time was either compound present in concentrations approaching those concomitantly measured for 6-TG. In our HPLC technique, 6-thioxanthine and 6-thioguanosine elute together. Although in plasma there was a peak due to one or both of these thiopurines, the concentrations were very small compared to that of the parent compound. In addition to these above known metabolites of 6-TG, we detected three unidentified metabolites, all of which were detected at 310 nm and none of which co-chromatographed with guanine, guanosine, 6-mercaptopurine, 6-mercaptopurine riboside or deoxyriboside, 6-methylthiouric acid, or 6-methylthioxanthine (Fig. 1). Plasma concentrations of all three metabolites rose slowly, and at later times in the studies approached or surpassed concomitant concentrations of 6-TG. Crystals of 6-TG and 6-thiouric acid were observed in the urine of all six patients from whom samples were obtained.

Discussion

In a previous phase I study [2], 6-TG given IV at doses of 400–600 mg/m^2 produced therapeutic responses with minimal toxicity. In view of a subsequent study by Presant et al. [9] it seemed logical to start with 700 mg 6-TG/m^2 and to escalate the dose until dose-limiting toxicities occurred. In our study seventeen patients received 6-TG doses of 700 mg/m^2 or greater and four patients had at least one course at 1,200 mg/m^2. The major toxicity observed was leukopenia, with the lowest WBC count nadir of 900/μl produced by a 1,200 mg/m^2 dose. Other toxicities observed were similiar to those reported for oral doses of 6-TG studies and included nausea, vomiting, anorexia, stomatitis and diarrhea [4, 5]. The toxicities seen with the high IV doses of 6-TG but not with the usual oral dose of 6-TG were renal dysfunction and rhinorrhea. Although renal dysfunction has been reported as a transient toxicity by Presant et al. [9], renal dysfunction may be a potentially serious and permanent complication. Among the six of our patients who underwent pharmacokinetic studies, crystalluria was observed in all urine samples. These urinary crystals were composed mainly of 6-TG and 6-thiouric acid. In that 6-TG and 6-thiouric acid are highly insoluble at a pH of 5–7, it seems plausible that they may precipitate in the renal parenchyma and, as such, may produce renal damage similar to uric acid nephropathy or 6-mercaptopurine nephropathy [3]. Transient, asymptomatic electrocardiographic changes, and transient episodes of palpation and hypotension have been observed in patients receiving intermittent high-dose IV 6-TG (M. Kovach, 1980, personal communication). Since all of our studies were performed on outpatients, no cardiac monitoring or serial electrocardiograms were obtained. However, we observed no signs of cardiac dysfunction and no patients complained of cardiac symptoms while on this study.

Our pharmacokinetic studies represent the first application of reverse-phase HPLC to the study of the behaviour in human plasma of 6-TG and its metabolites. As might be expected, there are several similarities between our results and the earlier study by LePage and Whitacre [7], who used ^{35}S-labeled 6-TG and paper chromatography to study 6-TG pharmacology in man. In our studies, administration of large IV doses of 6-TG produced peak plasma concentrations that were roughly 6 times greater than those reported by LePage and Whitacre [7] in a patient treated IV with 135 mg ^{35}S-TG/m^2 or about one-sixth the dose given to our patients. On the other hand, there are several important differences between the two studies. Whereas LePage and Whitacre [7] reported that parent compound represented a minor percentage of the total plasma 6-TG-derived radiolabel, our studies reveal 6-TG to be the major thiopurine present for at least the first 8 h after administration and to account for greater than 50% of thiopurines measured during that time. In addition, 6-TG persisted longer in plasma in our studies than it did in those of LePage and Whitacre [7], who reported a median $t_{1/2}$ of 80 min with individual values ranging from 25 to 240 min. It may well be that these quantitative differences reflect the large amounts of 6-TG administered in our studies, which could conceivably saturate the pathways for 6-TG metabolism and elimination. This could allow the attainment of higher concentrations of parent compound without concomitantly increased concentrations of metabolites, and also allow 6-TG to persist in plasma for longer periods of time. In addition to these quantitative differences in 6-TG pharmacology, our studies differ in several qualitative respects from earlier reports. As expected, at early times after 6-TG infusion, we observed significant plasma concentrations of 6-thiouric acid and 6-methyl TG, both of which are recognized major metabolites of 6-TG. Our

methodology also revealed the presence, early after 6-TG administration, of relatively large amounts of 6-thioxanthine or 6-thioguanosine and the gradual accumulation of relatively large amounts of three as yet undefined materials. These three substances are clearly related to 6-TG therapy and while they may, in fact, reflect the accumulation of some normal tissue constituent, their elution pattern and detection at 310 nm make it likely that they are 6-TG metabolites bearing a methylated thiol group. Whether the presence of these materials reflects saturation of the normal 6-TG metabolizing pathways and utilization of alternate pathways is as yet unknown. We are currently attempting to resolve this question as well as to identify definitively the structures of unknowns 1, 2, and 3.

Although the dose schedule used in this study was not escalated to maximal toxicity, some toxicity, especially transient renal dysfunction, occurred. Furthermore, there were no objective responses among our patients though higher doses were used than in the previous phase I study. We therefore conclude that IV intermittent high-dose 6-TG has little activity in colorectal cancer and further trials do not seem warranted.

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