A Phase I and Pharmacokinetic Study of the Mitochondrial-specific Rhodacyanine Dye Analog MKT 077


ABSTRACT

This Phase I study was performed to evaluate the tolerability and pharmacokinetic behavior of MKT-077, a water-soluble rhodacyanine dye analogue, which partitions into tumor cell mitochondria where it is thought to act as a metabolic poison, leading to G1 arrest and apoptosis. Thirteen patients with advanced solid malignancies were treated with MKT-077 administered as a 30-min i.v. infusion weekly for 4 weeks every 6 weeks at doses ranging from 42 to 126 mg/m²/week. The principal toxicity was renal magnesium wasting, which was dose-limiting (grade 3) in one patient at each of the 84- and 126-mg/m² dose levels. The other three patients at the 126-mg/m² dose level developed grade 2 hypomagnesemia, which was cumulative in nature, improved with i.v. magnesium supplementation, and was controlled in two patients by the administration of prophylactic magnesium before and after treatment with MKT-077. Given the requirement for extensive monitoring of serum magnesium before and after treatment with MKT-077, the toxicity profile was consistent with the preferential accumulation of the agent within tumor cell mitochondria, and biologically relevant plasma concentrations were achieved.

INTRODUCTION

The rhodacyanine analogue MKT-077, also known as 1-ethyl-2-[(3-ethyl-5-(3-methylbenzothiazolin-2-ylidenylidene)methyl}pyridinium chloride, localizes in the mitochondria and induces G1 arrest and apoptosis (1–3). Cationic rhodacyanine dyes, which were initially developed for use in the photographic industry, partition into mitochondria in response to the mitochondrial transmembrane potential (4–9). This unique characteristic led to their usefulness as dyes in fluorescence microscopy for localization of mitochondria and quantification of the mitochondrial transmembrane potential (4, 5). Inherent differences in the mitochondrial transmembrane potential between malignant epithelial cells and normal cells contribute to the selective accumulation of rhodacyanine dyes in the mitochondria of malignant cells (6–8, 10), suggesting a role for this class of compounds in the treatment of malignancies.

MKT-077 produces structural and functional alterations in the mitochondria of malignant cells (11), triggering the apoptotic cascade specifically in cells that accumulate the agent (2). The differential behavior of malignant and nonmalignant cells following treatment with MKT-077 has also been demonstrated in vitro (11). In one such experiment, mitochondria of human CRL-1420 pancreatic carcinoma cells became abnormally enlarged, with a loss of cristae and dense matrix inclusions following treatment with 2 μg/ml of MKT-077 for 20 h, whereas no such ultrastructural changes occurred in CV-1 kidney epithelial cells treated with an identical exposure to MKT-077 (11). In another study, MKT-077 inhibited mitochondrial aerobic metabolism in human CX-1 colon carcinoma cells at a concentration that was one quarter of that required to produce the same effect in normal CV-1 kidney epithelial cells (11). Additionally, treatment with 3 μg/ml of MKT-077 for 24 h resulted in the degradation of the mitochondrial DNA of CX-1 and CR-1420 cells, but no degradative effects on the mitochondrial DNA of normal CV-1 cells were noted (11). Photoirradiation potentiated the mitochondrial toxicity of MKT-077 (12). The cumulative results of metabolic studies with MKT-077 suggest that the agent causes dissipation of the mitochondrial transmembrane potential, which is regarded as an early step in the apoptotic cascade (13–15). It has been demonstrated that after collapse of the mitochondrial transmembrane potential, mitochondria release proapoptotic factors into the cytoplasm, which either di-
rectly or indirectly induce nuclear degradation and cell death (13–15).

The specificity of MKT-077 for the mitochondria of malignant cells has been associated with selective cytotoxicity against malignant cells in vitro. In the human CX-1 colon, MCF-7 breast, CRL-1420 pancreas, EJ bladder, and LOX melanoma tumor cell lines, the IC_{50} values of MKT-077 ranged from 0.15 to 0.5 μg/ml, whereas the IC_{50} value of MKT-077 in CV-1 epithelial cells was 40 μg/ml (1). Treatment with 0.25–4 μg/ml of MKT-077 for 1–5 days induced cell death in human DU-145 and PC-3 prostate cancer cells, whereas NPF-209 and NF-2 fibroblast cells remained viable after treatment with MKT-077 on identical schedules (16). In fresh surgical specimens taken directly from patients, the mean (± SD) IC_{50} values of MKT-077 were 8.4 ± 4.6 μg/ml and 66.5 ± 37.7 μg/ml in gastric cancer cells and normal spleen cells, respectively (17). Additionally, MKT-077 inhibited the growth of clonogenic cells from breast, ovary, endometrial, colon, and non-small cell lung cancer specimens in the human tumor cloning assay (18).

MKT-077 has also been demonstrated to inhibit the growth of human tumor xenografts (1, 19). The i.v. administration of 7.5 mg/kg of MKT-077 every other day for 2 weeks inhibited the growth of human A-498 renal carcinoma xenografts, and the i.v. administration of 11.25 mg/kg of MKT-077 repeatedly over 20 days inhibited the growth of DU-145 prostate xenografts (1). In addition, the continuous s.c. infusion of 20 mg/kg/day for 7 days inhibited the growth of human St-4 gastric, Co-4 colon, and CRL-1420 pancreatic carcinoma xenografts (19). Finally, after the administration of 5 mg/kg/day of MKT-077 for 5 days, the median survival of male nude mice bearing peritoneal LOX melanoma xenografts was increased 3-fold compared to untreated controls (1).

In the toxicological evaluations of MKT-077 in vivo, the principal effects were renal in nature (2). After daily treatment with MKT-077 for 5 days, rodents and dogs developed reddish orange-stained urine, elevated blood urea nitrogen and serum creatinine concentrations, and histopathological changes consistent with acute papillitis, chronic interstitial nephritis, and tubular necrosis (2). Although chronic interstitial nephritis, which was present in both treated and control animals, persisted during the recovery period, other biochemical and histopathological changes indicative of nephrotoxicity were reversible (2). Furthermore, the administration of 10% mannitol both before and after treatment with MKT-077 on days 1–5 protected treated animals from nephrotoxicity (2). Other common effects included anorexia, emesis, and decreased food consumption (2). Although rhodacyanine dyes have been shown to accumulate within cardiac myocytes (10), cardiac effects were not observed in preclinical studies of MKT-077 (2).

The rationale to develop MKT-077 was based on its ability to selectively accumulate within the mitochondria of malignant cells and induce apoptosis, as well as its acceptable toxicity profile in animals. The principal objectives of this Phase I and pharmacological study were to: (a) describe the principal toxicities of MKT-077 administered as a 30-min i.v. infusion weekly for 4 weeks every 6 weeks in patients with advanced solid malignancies; (b) determine the maximum tolerated dose of MKT-077 on this schedule; (c) characterize the pharmacokinetic profile of MKT-077; and (d) seek preliminary evidence of antitumor activity in patients with advanced cancers.

**PATIENTS AND METHODS**

**Patient Selection.** Patients with histologically confirmed advanced solid malignancies refractory to standard therapy or for whom no effective therapy existed were candidates for this study. Other eligibility criteria included: (a) age ≥18 years; (b) Karnofsky performance status of at least 70% (ambulatory and capable of self-care); (c) life-expectancy of at least 12 weeks; (d) presence of measurable or evaluable disease; (e) no known untreated brain metastases; (f) no chemotherapy or radiotherapy in the previous 4 weeks; (g) no nitrosoureas or mitomycin C within the previous 6 weeks; (h) no investigational drug in the previous 4 weeks; (i) no active uncontrolled infection; (j) adequate hematopoietic (absolute neutrophil count ≥1500/μl, platelet count ≥100,000/μl, and hemoglobin ≥9.0 g/dl), hepatic (total serum bilirubin ≤1.5 times the upper limit of normal, and transaminases ≤2 times the upper limit of normal in the absence of liver metastases and ≤5 times the upper limit of normal in the presence of liver metastases), and renal (blood urea nitrogen ≤20 mg/dl, serum creatinine concentration ≤1.4 mg/dl, and estimated creatinine clearance ≥60 ml/min) functions; (k) prothrombin time and activated partial thromboplastin time ≤1.5 times the upper limit of normal; (l) LVEF of at least 50% as determined by radionuclide ventriculography; and (m) no other coexisting medical problems of sufficient severity to prevent full compliance with the study. Females of childbearing age were required to be practicing effective contraceptive measures and must have had a negative serum pregnancy test before study entry. Written informed consent was obtained according to federal and institutional guidelines.

**Drug Dosage and Escalation.** The starting dose of MKT-077 was 42 mg/m² administered as a 30-min i.v. infusion weekly for 4 weeks every 6 weeks. This starting dose was based on a Phase 1 clinical trial in which patients received MKT-077 as a 30-min i.v. infusion at doses of up to 48 mg/m² on days 1, 3, and 5 every 28 days without experiencing any DLT (1). For the present Phase I trial, MKT-077 doses were to be escalated in groups of new patients to 56, 84, and 126 mg/m² and by 33% increments thereafter. It was planned that at least three patients would be enrolled at each dose level. Intraindividual dose escalations were permitted if the cohort of patients at the next dose level had completed at least two courses of therapy without experiencing DLT.

DLT was defined as any one of the following: (a) grade 4 hematological toxicity, consisting of either absolute neutrophil count <500/μl, platelet count <25,000/μl, or hemoglobin <6.5 g/dl; (b) grade 2 renal toxicity, consisting of serum creatinine ≥1.5 times the upper limit of normal, blood urea nitrogen ≥11 g/deciliter, gross hematuria, or proteinuria ≥3 g/liter; (c) and any nonrenal nonhematological toxicity that was at least grade 3 in severity. If one episode of DLT occurred, a maximum of six

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3The abbreviations used are: LVEF, left ventricular ejection fraction; DLT, dose-limiting toxicity; HPLC, high-performance liquid chromatography; AUC, area under the serum concentration-versus-time curve.
patients were treated at that dose level. The maximum tolerated dose was defined as the highest dose in which less than two of six new patients experienced DLT. Toxicities were graded according to the National Cancer Institute common toxicity criteria (20).

Patients who had not developed progressive disease at the time of reassessment and who had not experienced grade $\geq 2$ renal toxicity or grade $\geq 3$ nonrenal toxicity were permitted to continue treatment with MKT-077 at the same dose level. In patients who had experienced grade 1 or 2 renal toxicity, treatment was delayed until the renal toxicity had fully resolved. Those patients who had experienced grade 1 renal toxicity were eligible for continuation of treatment with MKT-077 at the same dose level, whereas those who had experienced grade 2 renal toxicity were eligible for continuation of treatment with a 25% dose reduction. All patients who were retreated after the resolution of renal toxicity were required to receive hydration consisting of 0.5 liters of 0.45% sodium chloride with 12.5 g of mannitol, which was to be administered both before and after the infusion of the study drug. MKT-077 was discontinued in patients who had experienced grade $\geq 3$ renal toxicity. Patients who had experienced grade 3 nonrenal toxicity were permitted to continue treatment with MKT-077 with a 25% dose reduction, whereas patients who had experienced grade 4 nonrenal toxicity were permitted to continue treatment with a 50% dose reduction.

Drug Administration. MKT-077 was obtained from Sandoz Research Institute (East Hanover, New Jersey) as an orange lyophilized powder for i.v. infusion. Each 30 mg/10 ml vial was reconstituted with 5 ml of sterile water for injection. The solution of MKT-077 was further diluted to the final concentration in 100 ml of 0.9% sodium chloride for injection. Because MKT-077 was known to completely decompose after 1 week in the light and because the agent did not decompose when stored in the dark, the container and the i.v. tubing were wrapped in aluminum foil to prevent exposure to the light (2). The final solution was administered i.v. immediately after mixing to ensure full potency.

MKT-077 was administered as a 30-min i.v. infusion weekly for 4 weeks every 6 weeks. Initially, patients did not receive hydration before or after treatment unless they had had exhibited grade 1 or 2 renal toxicity attributable to MKT-077 during a prior course. However, the scheme was modified when preclinical studies in rodents demonstrated reduced renal toxicity with mannitol diuresis (2). Beginning at the 84 mg/m$^2$ dose level, patients received 500 ml of 0.45% sodium chloride with 12.5 g of mannitol over 1 h both before and after treatment with MKT-077. No antiemetic medications were administered prophylactically.

Pre-treatment and Follow-up Studies. Before each course of treatment, histories and physical examinations were performed, and the following evaluations were obtained: complete blood counts, differential WBC counts, routine chemistry and electrolyte tests, clotting studies, urinalyses, 24-h urine collections for total protein, electrocardiograms, chest radiographs, and appropriate tumor markers. Histories, physical examinations, complete blood counts, routine chemistry and electrolyte tests, urinalyses, and toxicity assessments were also obtained weekly. Radionucleotide ventriculography was performed before enrollment and after every two courses of therapy.

Appropriate radiological studies for documentation of measurable disease were performed before enrollment and after every two courses of therapy. A complete response was scored if there was disappearance of all known disease on two measurements, separated by a minimum of 4 weeks. A partial response required at least a 50% reduction in the sum of the products of the bidimensional measurements, separated by at least 4 weeks. Progressive disease was defined as an increase in the sum of the products of the bidimensional measurements of all known disease by at least 25% or the appearance of new lesions.

Pharmacokinetic Sampling and Assay. To determine the pharmacokinetic behavior of MKT-077 and its hydroxylated metabolite FJ-934, whole blood samples were drawn from all patients during their first course of treatment. Blood was sampled immediately before the infusion, at 10 and 20 min into the infusion, at the end of the infusion, and then at 1, 2, 4, 8, 16, 24, 48, and 72 h after infusion. Blood was also sampled on days 8, 15, and 22 after infusion. The 10-ml samples were collected in serum separation tubes, which had been wrapped in aluminum foil to prevent exposure to the light. After clotting for 30 min, the serum was separated by centrifugation at 1000 $\times$ g for 10 min. After centrifugation, the serum was transferred to polypropylene screw-cap tubes, which had been covered in aluminum foil and then stored in the dark at $-70^\circ$C.

MKT-077 and FJ-934 concentrations were measured by reversed-phase HPLC. Analytical standards were provided by Fuji Photo Film, Co. (Kanagawa, Japan). Before quantitation, the samples were thawed on ice, and a 0.5-ml aliquot was transferred to a clean 5-ml tube. One hundred microliters of internal standard were added to the sample, and MKT-077 and FJ-934 were extracted using 2 ml of acetonitrile. After centrifugation, the organic layer was transferred to a clean glass tube and dried under nitrogen. Extracts were reconstituted in 300 $\mu$l of HPLC mobile phase (acetonitrile: 0.1% aqueous trifluoroacetic acid adjusted to pH 2.5, 38:62, v/v). All reconstituted samples were kept in the dark at 5°C before injection onto the HPLC column.

A Shimadzu Series 10A HPLC system (Columbia, MD) was used, and data were collected using a Multichrom 2 Data Acquisition System (Fisons, Beverly, MA). After injection of a 100-ml sample, the compounds were separated using a 5-$\mu$m, 150 $\times$ 4.6-mm TSK-Gel ODS-80TM column (Tosohas, Montgomeryville, PA) maintained at 40°C. Detection was at 490 nm, and the flow rate was 0.8 ml/min. The retention times for MKT-077, FJ-934, and the internal standard were 20.9, 7.7, and 10.1 min, respectively.

Drug concentrations were calculated from calibration curves at MKT-077 concentrations that ranged from 1.30 to 2100 ng/ml and FJ-934 concentrations that ranged from 0.69 to 1100 ng/ml. Unknown concentrations of MKT-077 and FJ-934 were determined using linear least-squares regression with a weighting factor of 1/x. The lower limit of assay quantification for MKT-077 was 1.3 ng/ml, whereas for FJ-934, it was 0.69 ng/ml.

In addition to MKT-077 and FJ-934 serum concentrations, urine concentrations were determined for one patient at the
42-mg/m² dose level. Pooled urine collections were obtained before the infusion and from 0 to 6 h, 6 to 24 h, and 24 to 48 h after infusion. A 20-ml aliquot was taken from each pooled collection and transferred to a sample storage tube, which was frozen at −70°C. To perform the urine assay, 0.5 ml of urine were applied to a conditioned C8 solid phase extraction disc. The cartridge was washed with 0.5-ml aliquots of water and methanol:water (40:60, v/v) and eluted with 0.5 ml of 20 mM ammonium acetate in methanol. The drying and reconstitution steps were the same as those described for the serum assay.

**Pharmacokinetic Analysis.** Individual MKT-077 serum concentrations were analyzed using model-independent methods (21). $C_{\text{max}}$ and $T_{\text{max}}$ were determined by inspection of the plasma concentration-versus-time curves, where $C_{\text{max}}$ is the maximum serum concentration and $T_{\text{max}}$ is the time of maximum serum concentration. The terminal rate constant, $k$, was calculated as the negative of the slope of the log-linear terminal portion of the concentration-versus-time curve using linear regression. The terminal half-life, $t_{1/2}$, was calculated as 0.693/$k$. The AUC from time zero to the time of the final quantifiable sample, $AUC(t_f)$, was calculated using the linear trapezoidal method and was extrapolated to infinity according to the following equation:

$$AUC_{\text{ss}} = AUC(t_f) + C(t_f)/k$$

where $C(t_f)$ was the estimated concentration at time $t_f$, $Cl_{\text{ss}}$, which is systemic clearance, was calculated by dividing the dose by $AUC_{\text{ss}}$. $V_{\text{ss}}$, which is the volume of distribution at steady state, was calculated using standard noncompartmental methods based on the statistical moment theory (21).

MKT-077 pharmacokinetic parameters were summarized using descriptive statistics. Statistical analysis was performed using the JMP version 3.1.6.2 statistical software program (SAS Institute, Cary, NC).

**RESULTS**

**General.** Thirteen patients, whose characteristics are detailed in Table 1, received 20 courses of MKT-077. The total numbers of patients and courses administered as a function of MKT-077 dose level and the overall dose escalation scheme are depicted in Table 2. Although there were no dose reductions, three patients at the 126-mg/m² dose level were unable to complete their final course of therapy. One patient with adenocarcinoma of unknown primary was found to have progressive disease on day 15 of course 2, and a second patient with a soft-tissue sarcoma was found to have progressive disease on day 15 of course 1. The third patient, a 54-year-old woman with a malignant carcinoid neoplasm, developed grade 3 hypomagnesemia on day 22 of course 2, for which treatment was held. There were no intrapatient dose escalations.

**Nephrotoxicity.** The principal toxicity of MKT-077 administered weekly for 4 weeks every 6 weeks was nephrotoxicity. The total number of courses associated with serum creatinine elevation, proteinuria, and hypomagnesemia attributable to MKT-077 are depicted in Table 3. Whereas no patients developed drug-related renal failure or significant proteinuria, five patients developed grade 2–3 hypomagnesemia, which was cumulative in nature. Serum magnesium levels were not routinely measured until one patient at the 84-mg/m² dose level was incidentally found to have grade 3 hypomagnesemia at the time of withdrawal from the study. Thereafter, hypomagnesemia, which was not typically associated with any other electrolyte abnormalities, was observed in all four patients at the 126-mg/m² dose level. Given the requirement for extensive monitoring of serum magnesium levels and the frequent administration of magnesium supplementation, dose escalation >126 mg/m² was not considered feasible.
One patient at each of the 84-mg/m² and 126-mg/m² dose levels developed dose-limiting renal magnesium wasting. The first patient, a 51-year-old man with hepatocellular carcinoma who had not previously received any nephrotoxic drugs or diuretics, developed grade 3 hypomagnesemia (0.6 mg/dl) after one course of treatment at the 84-mg/m² dose level. The hypomagnesemia was discovered as he was removed from the study on day 39 of course 1 for progressive disease. Over the following 2 weeks, his hypomagnesemia persisted, and he required a total of six doses of 2–4 g of i.v. magnesium sulfate. The second patient to develop grade 3 hypomagnesemia was a 54-year-old woman with a malignant carcinoid neoplasm who was treated with MKT-077 at the 126-mg/m² dose level. This patient’s serum magnesium was not measured before treatment, and she was found to have grade 3 hypomagnesemia (0.6 mg/dl) and grade 2 hypocalcemia (6.6 mg/dl) on day 22 of course 2. She had been previously treated with carboplatin and streptozotocin and had been receiving 20 mg daily of oral furosemide for the treatment of peripheral edema beginning on day 9 of course 1. This patient received 2–6 g of i.v. magnesium sulfate almost daily for 3 weeks concurrently with oral magnesium 80 meq/day. On day 43 of course 2, she was withdrawn from the study for progressive disease, and 1 month later, she continued to require oral magnesium supplementation.

The last three patients enrolled at the 126-mg/m² dose level, for whom serum magnesium concentrations were normal before treatment with MKT-077, developed hypomagnesemia, which reached grade 2 in severity. The hypomagnesemia appeared to be cumulative in nature, improved with i.v. magnesium supplementation, and was controlled in two patients by the intermittent administration of furosemide for the treatment of peripheral edema beginning on day 9 of course 1. This patient received 2–6 g of i.v. magnesium sulfate almost daily for 3 weeks concurrently with oral magnesium 80 meq/day. On day 43 of course 2, she was withdrawn from the study for progressive disease, and 1 month later, she continued to require oral magnesium supplementation.

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The characteristics of the hypomagnesemia were best documented in a 61-year-old man who had previously received two courses of carboplatin and one course of cisplatin for the treatment of metastatic adenocarcinoma of unknown origin involving the mediastinum. This patient developed hypomagnesemia with concurrent magnesuria as demonstrated in Fig. 1, which depicts the patient’s serum magnesium and 24-h urine magnesium as a function of time during his four courses of MKT-077. While on study, the patient’s other urine electrolytes, including phosphorus and calcium, were within normal limits. The interpretation of his urine magnesium levels was confounded by the concurrent administration of 2 g of i.v. magnesium sulfate with each dose of MKT-077 beginning on day 8 of course 1 and by the intermittent administration of furosemide and thiazide diuretics for the treatment of fluid retention beginning on day 9 of course 3. However, the elevated 24-h urine magnesium levels in the presence of persistent hypomagnesemia suggested that magnesium was inappropriately excreted by the kidneys.

Miscellaneous Toxicities. Peripheral edema was the most common nonrenal toxicity attributable to treatment with MKT-077. Mild to moderate (grade 1–2) peripheral edema was noted in 7 of 20 courses, involving 1 course in one patient at the 42-mg/m² dose level and 6 courses in two patients at the 126-mg/m² dose level. The edema involved the extremities and/or face. There was no evidence of pulmonary edema or hypoaalbuminemia. Both patients at the 126-mg/m² dose level who developed peripheral edema also developed grade 2 weight gain requiring treatment with oral furosemide. Routine hydration administered on days 1–5 at 126 mg/m² possibly contributed to the peripheral edema seen at this dose level.

Nausea and vomiting attributable to MKT-077 were associated with five courses in four patients across all dose levels. Although patients did not routinely receive prophylactic antiemetics, those four patients who experienced grade 1–2 nausea and vomiting after their first treatment with MKT-077 were premedicated with either phenothiazines or serotonin antagonists before subsequent treatments. These antiemetics prevented additional nausea and vomiting in all but one patient who developed grade 3 nausea and vomiting on day 1 of course 2 at the 126-mg/m² dose level. This toxicity was not considered to be dose-limiting because the patient tolerated additional doses of MKT-077 administered in combination with 3 days of oral serotonin antagonists.

Three of the four patients who received at least two courses of MKT-077 completed sequential radionucleotide ventriculography studies and were fully evaluable for measuring the effects of MKT-077 on left ventricular function. One patient at the 48-mg/m² dose level who had previously received doxorubicin and mediastinal radiation for treatment of his malignant thymoma developed an asymptomatic transient decline from his pretreatment LVEF exceeding 20%. The LVEF was 59% at
baseline, 42% on day 32 of course 2, and 59% on day 38 of course 2. At the 126-mg/m² dose level, one patient developed a 9% decline in ejection fraction after two courses, and a second patient developed a 14% decline in ejection fraction after four courses. As an additional screen for cardiac toxicity, all patients on study completed serial serum sampling for creatinine kinase, and only one patient at the 48-mg/m² dose level developed a transient grade 1 elevation.

Hematological toxicity was rare. Myelosuppression was observed in one patient who developed grade 1 thrombocytopenia and grade 1 neutropenia during her second course of therapy at the 126-mg/m² dose level. This patient had a carcinoid malignancy for which she had received prior chemotherapy consisting of three courses of carboplatin and etoposide, four courses of doxorubicin, and two courses of streptozotocin. Anemia resulting in at least a 2% decrement in hematocrit value was observed in 16 courses (80%), and a >4% decrement in hematocrit value was observed in 12 courses (60%). One patient with a primary adenocarcinoma of unknown origin who had previously received two courses of carboplatin and paclitaxel and one course of cisplatin required a transfusion of RBCs for grade 3 anemia that was possibly attributable to treatment with MKT-077.

Several other toxicities were potentially related to treatment with MKT-077. One patient with melanoma experienced grade 3 diarrhea for 24 h on day 16 of course 1 at the 84-mg/m² dose level. Two other patients at the 42- and 56-mg/m² dose levels each developed grade 1 diarrhea during their first course of therapy. Other rare toxicities possibly attributable to MKT-077 included grade 1–2 fatigue (three courses), headache (three courses), fever (two courses), rigors (two courses), and paraesthesias of the hands and mouth (two courses).

Pharmacological Studies. Blood sampling to evaluate the pharmacokinetic behavior of MKT-077 was performed in all 13 patients. A representative concentration-versus-time curve for a patient treated with MKT-077 at the 126-mg/m² dose level is shown in Fig. 2, and the pertinent pharmacokinetic parameters of MKT-077 as a function of dose level are listed in Table 4. MKT-077 exhibited a large Vₘₐₓ, averaging 685 ± 430 liters/m². Systemic clearance averaged 39 liters/h/m², with an overall interpatient variability (coefficient of variation) of 32%, and the mean elimination 1/2 was 37 ± 17 h. In many of the samples drawn just before treatment on days 8, 15, and 22, MKT-077 was still detectable in the serum. Within the narrow dose range studied, drug exposure, as measured by Cₘₐₓ and AUC, increased linearly in proportion with dose (r² = 0.41 and 0.51, respectively). At the 126-mg/m² dose level, the Cₘₐₓ was 6273 ± 5320 ng/ml, and the AUCₙₐ was 3760 ± 2122 ng·h/ml. The serum concentration of the FJ-934 metabolite represented <0.1% of the parent compound and was below the assay limit of quantification at most sample time points in the majority of patients. In the one patient for whom urine MKT-077 and FJ-934 concentrations were determined, 8.3% of the total MKT-077 dose was recovered as the parent drug, and 0.3% was recovered as the FJ-934 metabolite in the urine collected over 48 h.

Among the patients in whom hypomagnesemia was observed, there was no apparent relationship between the severity of hypomagnesemia and drug clearance. However, because serum magnesium levels were not routinely measured at the first three dose levels, a complete pharmacodynamic assessment of hypomagnesemia was not possible.

**DISCUSSION**

Cationic rhodacyanine dyes are a unique class of compounds that partition across mitochondrial membranes and trigger the apoptotic cascade (1–9). In response to a high mitochondrial transmembrane potential, rhodacyanine dyes accumulate within the mitochondria of malignant cells (3–9) and inhibit mitochondrial aerobic metabolism (7, 9, 11, 22, 23). This may result in the collapse of the mitochondrial transmembrane potential, an event that precedes the nuclear changes characteristic of apoptosis (13–15). In experimental systems, proapoptotic stimuli initiate permeability transition, allowing an equilibration of ions within the matrix and intermembrane space of the mitochondria (13–15). Ostensibly, any major change in energy balance, such as that produced by rhodacyanine dyes, may provoke permeability transition (14). After dissipation of the mitochondrial transmembrane potential, mitochondria release factors that induce apoptosis through caspase-dependent and caspase-independent pathways (13–15).

MKT-077, a water soluble rhodacyanine dye analogue, was selected for clinical development because it perturbs the structural and functional integrity of mitochondria in neoplastic cells, resulting in significant anticancer activity both in vitro and in vivo (1, 3, 11, 16–19). This Phase I study was designed to evaluate the feasibility of administering MKT-077 weekly for 4 weeks every 6 weeks to patients with solid malignancies. The principal toxicity of this regimen was hypomagnesemia. Although grade 3 hypomagnesemia was first demonstrated in a patient treated at the 84-mg/m² dose level, this effect was not recognized as a drug-related toxicity until it consistently developed in patients treated at the 126-mg/m² dose level. Given the requirement for extensive monitoring of serum magnesium levels, dose escalation >126 mg/m² was not considered feasible. However, the 126-mg/m² dose level was well-tolerated with magnesium supplementation, and it is therefore recommended.

![Fig. 2](clinicalcancerres.aacrjournals.org) Representative MKT-077 serum concentration-versus-time curve at the 126-mg/m² dose level.
for subsequent disease-directed evaluations of MKT-077 on this schedule.

Hypomagnesemia may result from a deficiency of magnesium in the diet, decreased intestinal absorption, or increased urinary excretion (24). Malnutrition did not appear to be the cause of hypomagnesemia in this study because there was no evidence of corresponding weight loss or hypoalbuminemia. In addition, there was no indication that the hypomagnesemia was due to decreased intestinal absorption because it was not associated with severe vomiting or diarrhea. Thus, the hypomagnesemia appeared to result from increased urinary excretion. Indeed, serum and urine magnesium concentrations in one patient who developed hypomagnesemia at the 126-mg/m² dose level were compatible with a state of inappropriate urinary magnesium excretion (>20 mg/day; Ref. 24). The previous administration of agents that induce renal tubular toxicity, such as cisplatin and streptozotocin, and the coadministration of diuretics may have predisposed some patients to renal magnesium wasting (24, 25). However, the occurrence of grade 3 hypomagnesemia in a patient with no predisposing factors indicates that MKT-077 alone is sufficient to produce renal toxicity.

The presence of renal magnesium wasting in the absence of other biochemical abnormalities suggests that MKT-077 prevents magnesium transport in the thick ascending limb of the loop of Henle, where 60% to 70% of the filtered magnesium is normally reabsorbed (24). Magnesium reabsorption is promoted by an electrochemical gradient created by the transport of sodium, chloride, and potassium (24). Filtered sodium, potassium, and chloride are reabsorbed by tubular cells via luminal transporters, and reabsorbed sodium is subsequently transported out of the tubular cells via basolateral pumps that exchange sodium for potassium (24). Potassium moves back into the lumen through luminal potassium channels, and chloride moves into the peritubular capillaries via basolateral chloride channels (24). With this movement of cationic potassium into the lumen and anionic chloride into the peritubular capillaries, the lumen becomes relatively electropositive, promoting the passive reabsorption of magnesium through the paracellular pathway between cells (24). Although it is not known how MKT-077 prevents magnesium reabsorption, studies in rodents have demonstrated that the drug accumulates in the kidneys (26) and produces ultrastructural changes in the mitochondria of the thick ascending limb of the loop of Henle (2). By inhibiting ATP production in this segment of the nephron, MKT-077 may interfere with the electrochemical gradient that facilitates tubular magnesium reabsorption.

It was initially proposed that MKT-077 would be toxic to vital tissues dependent on the mitochondrial energy supply based on the agent’s ability to inhibit mitochondrial aerobic metabolism in vitro (11). In an attempt to discern the effect of MKT-077 on vital organs, mitochondrial function was examined in rats following the administration of the maximally tolerated dose of 15 mg/kg/day for 5 days (27). In contrast to electron microscopy studies that demonstrated ultrastructural changes in the mitochondria of rat kidneys (2), mitochondrial aerobic metabolism and mitochondrial DNA levels in rat kidneys appeared to be unaffected by treatment with MKT-077 (27). Furthermore, although mitochondrial aerobic metabolism was temporarily impaired in the rat liver, mitochondrial function was unaffected in the rat heart (27). Despite these in vivo results, patients receiving MKT-077 were monitored for cardiac toxicity because in vitro studies with another rhodacyanine dye, rhodamine 123, demonstrated retention within cardiac myocytes (10) and cessation of rhythmic cardiac myocyte pulsation (28). However, no patient in this Phase I study of MKT-077 developed symptomatic cardiac dysfunction. Another potential toxicity of agents that interfere with mitochondrial function and one that was observed in the Phase I trial of the antimitochondrial agent terephthalamide is asthenia (29). Nevertheless, patients receiving MKT-077 did not develop weight loss or decreased performance status, indicating that treatment with MKT-077 did not result in nonspecific toxicity against normal tissues.

Pharmacokinetic studies revealed a low $C_{\text{max}}$ (39 ± 13 liters/h/m²) and large $V_{ss}$ (685 ± 430 liters/m²). The MKT-077 $C_{\text{max}}$ values (1.2 ± 0.31 to 6.3 ± 5.3 µg/ml) exceeded IC₅₀ concentrations required for human CX-1 colon, MCF-1 breast, CRL-1420 pancreas, EJ bladder, and LOX melanoma tumor cell lines in vitro (0.15 to 0.5 µg/ml). In the one patient in whom urine MKT-077 concentrations were determined, only 8.3% of the total dose was excreted in the urine. This is consistent with pharmacological studies in rats, which demonstrated that MKT-077 was primarily excreted in the feces (26). Finally, plasma concentrations of the hydroxylated metabolite FJ-934 were minimal, indicating that FJ-934 is not a major metabolite.

The results of this Phase I study indicate that 126 mg/m² of MKT-077 administered weekly for 4 weeks every 6 weeks is well tolerated and results in manageable hypomagnesemia. MKT-077 represents a new class of anticancer agents, which preferentially accumulate within the mitochondria of malignant cells, inhibit mitochondrial aerobic metabolism, and induce nuclear apoptosis. The specificity of rhodacyanine dyes for malignant cells, coupled with their unique mechanism of action,

<table>
<thead>
<tr>
<th>MKT-077 dose level (mg/m²)</th>
<th>No. of patients</th>
<th>$C_{\text{max}}$ (ng/ml)</th>
<th>$V_{ss}$ (liters/m²)</th>
<th>$Cl_s$ (liters/h/m²)</th>
<th>$t_{1/2}$ (h)</th>
<th>AU/C₅₀ (ng/h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>3</td>
<td>1249 ± 310</td>
<td>945 ± 524</td>
<td>40 ± 7.3</td>
<td>46 ± 30</td>
<td>1059 ± 194</td>
</tr>
<tr>
<td>56</td>
<td>3</td>
<td>2457 ± 1069</td>
<td>612 ± 500</td>
<td>42 ± 19</td>
<td>28 ± 6.7</td>
<td>1611 ± 954</td>
</tr>
<tr>
<td>84</td>
<td>3</td>
<td>4150 ± 2149</td>
<td>623 ± 546</td>
<td>35 ± 9.3</td>
<td>32 ± 14</td>
<td>2532 ± 666</td>
</tr>
<tr>
<td>126</td>
<td>4</td>
<td>6273 ± 5320</td>
<td>576 ± 395</td>
<td>41 ± 20</td>
<td>35 ± 13</td>
<td>3760 ± 2122</td>
</tr>
<tr>
<td>All patients</td>
<td>13</td>
<td>865 ± 430</td>
<td>39 ± 13</td>
<td>37 ± 17</td>
<td></td>
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</tr>
</tbody>
</table>
indicates promise for these compounds in the treatment of malignancies.

REFERENCES


Clinical Cancer Research

A Phase I and Pharmacokinetic Study of the Mitochondrial-specific Rhodacyanine Dye Analog MKT 077