MKT-077, a Novel Rhodacyanine Dye in Clinical Trials, Exhibits Anticarcinoma Activity in Preclinical Studies Based on Selective Mitochondrial Accumulation

Keizo Koya, Yang Li, Hong Wang, Toshinao Ukai, Noriaki Tatsuta, Masayuki Kawakami, Tadao Shishido, and Lan Bo Chen

Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115 [K. K., Y. L., H. W., L. B. C.], and Ashigara Research Laboratories, Fuji Photo Film Co., Ltd., Minamiashigara, Kanagawa, 250-01, Japan [T. U., N. T., M. K., T. S.]

ABSTRACT

MKT-077 (formerly known as FJ-776) is a newly synthesized, highly water-soluble (>200 mg/ml) rhodacyanine dye that exhibits significant antitumor activity in a variety of model systems. In culture, MKT-077 inhibits the growth of five human cancer cell lines (colon carcinoma CX-1, breast carcinoma MCF-7, pancreatic carcinoma CRL-1420, bladder transitional cell carcinoma EJ, and melanoma LOX) but not monkey kidney CV-1, an indicator cell line for normal epithelial cells. In nude mice, MKT-077 inhibits the growth of s.c. implanted human renal carcinoma A498 and human prostate carcinoma DU145 and prolongs the survival of mice bearing i.p. implanted human melanoma LOX (tumor control = 344%). Subcellular localization indicates that MKT-077 is taken up and retained by mitochondria, and flow cytometric analysis suggests that CX-1 cells take up MKT-077 to a much greater extent than CV-1 cells. Quantitation of MKT-077 uptake by ethanol extraction shows that CX-1 cells accumulate 65-fold more MKT-077 than do CV-1 cells. MKT-077 is the first delocalized lipophilic cation with a favorable pharmacological and toxicological profile in preclinical studies. MKT-077 is now being investigated in Phase 1 clinical trials.

INTRODUCTION

Epithelial tumors, which constitute more than 85% of human cancers, are the major cause of death in the United States. Unfortunately, conventional chemotherapy, which is responsible for the cure of childhood leukemias, has met only limited success in the treatment of carcinomas. It underscores an urgent need for novel anticancer therapy that exploit hitherto untapped molecular targets and unexplored mechanisms.

Considerable efforts have been made to develop DLCs into potential anticancer agents (1–13). DLCs are accumulated by mitochondria in response to plasma and mitochondrial membrane potentials (negative inside; Refs. 1–3). Since carcinoma cells, particularly those of human origin, display an increased mitochondrial membrane potential when compared with normal epithelial cells (1), DLCs have been proposed as potential anticancer agents. DLCs explored previously for this purpose include rhodamine 123 (4–6), dequalinium (7, 8), N'-biss(2-ethyl-l,3-dioxolane)-krytocyanine (9), Victoria blue BO (10), tetraphenylphosphonium (11), thiacarbocyanines (12), and thiopyrylium AA-l (13). Although many of these compounds exhibit significant antitumor activity in culture and/or animal models, none of them have met the stringent criteria required for clinical development, in particular, with respect to water solubility, untoward effects, and pharmacokinetics.

In an effort to identify DLCs suitable for clinical trials, we have undertaken a screening program to examine as many of the 200,000 DLCs registered in the Chemical Abstract as is possible. A wide variety of DLCs from a library of more than 100,000 organic compounds at Fuji Photo Film Co., Ltd., has been screened and chosen. Rhodacyanine dyes, well-known in the photographic industry as sensitizers for silver halides (14), were found to be unique. They exhibit marked anticancer activity with relatively low toxicity. Upon their identification, we designed and synthesized over 1000 rhodacyanines, with careful consideration taken regarding structure-activity relationships. We examined characteristics including solubility, stability, toxicity, pharmacokinetics, and antitumor activity in culture and in nude mice. Based on this extensive study, the best rhodacyanine, MKT-077 (formerly known as FJ-776), was chosen for further characterization. MKT-077 is a highly water-soluble (>200 mg/ml) lipophilic compound that displays significant anticancer activity. MKT-077, as the first DLC with a favorable pharmacological and toxicological profile in preclinical studies, has been chosen for clinical development.

MATERIALS AND METHODS

MKT-077, 1-ethyl-2-[[3-ethyl-5-(3-methylbenzothiazolin-2-yliden)-4-oxothiazolidin-2-ylidenemethyl]pyridinium chloride (MKT-077, formerly known as FJ-776; C11H22N3OS2Cl, molecular weight 432.01), was chemically synthesized. The structure was determined by X-ray analysis of its crystal and various spectroscopic data. 1H-nuclear magnetic resonance, 13C-nuclear magnetic resonance, infrared, and mass. The peak temperature from the DSC spectrum was 261.3°C. MKT-077 is orange-colored (Amax 495 nm) and highly soluble in water (> 200 mg/ml). It is very stable and can be stored even in aqueous solution at 4°C for more than 1 year. The purity of MKT-077 used in this study was >99%, as determined by high-pressure liquid chromatography analysis. For cell culture studies, MKT-077 at 1 mg/ml in DMSO was prepared as a stock solution. For animal studies, MKT-077 was dissolved in 5% dextrose at 10 mg/ml and sonicated for 5 min in a bath sonicator (Branson; Bronson Ultrasorons Corp., Danbury, CT). All MKT-077 solutions were protected from light and stored at 4°C. Concentrations of MKT-077 were determined by absorption spectra to avoid weighing errors.

Cell Cultures. All cell lines were grown in a 50:50 mix of DMEM and RPMI 1640 (GIBCO Laboratories, Grand Island, NY) supplemented with 10% calf serum (Hyclone Laboratories, Inc., Logan, UT) and antibiotics at 37°C under 5% CO2, 95% air and 100% humidity. CRL-1420 (human pancreatic carcinoma) and CV-1 (normal African green monkey kidney) were from the American Type Culture Collection (Rockville, MD). CX-1 (human colon carcinoma) and LOX (human melanoma) were from Dr. M. Wolpert (National Cancer Institute, Bethesda, MD). EJ (human bladder transitional cell carcinoma) was from Dr. R. C. C. Willems (University of Michigan, Ann Arbor, MI). Clonogenic-assay and Measurement of Growth Rate. For the clonogenic assay, cells were seeded at 2000 cells/well in 6-well plates (Becton Dickinson Labware, Lincoln Park, NJ). On the following day, cells were treated with MKT-077, cisplatin (from Bristol-Meyer), or Adriamycin (Adria Lab) at varying concentrations in 2.5 ml of culture medium for 24 h. After rinsing, cells were incubated in drug-free medium for 1–2 weeks. Colonies were stained with 2% crystal violet in 70% ethanol and counted by an automated colony counter (Artrek counter model 880; Dynatech Laboratory, Inc., Chantilly, VA). The assay was performed in duplicate. For the inhibition of cell growth in mass cultures, cells were seeded on day 0 at 1 × 104 cells/100-mm dish, followed by the daily addition of MKT-077 at 3 µg/ml on day 1, 2, 3, and 4.
days 1–4. On each day, cells were trypsinized, and trypan blue-negative cells were counted by a hemacytometer.

**Time-Lapse Videomicroscopy.** Cells were seeded at $5 \times 10^5$ in a 60-mm dish, placed on a Leitz inverted microscope equipped with a chamber equilibrated with continuous infusion of 10% CO$_2$ and kept at 37°C. Phase-contrast images were captured by a Newvicon videocamera and recorded on a Panasonic Super VHS time-lapse videorecorder. MKT-077 was added daily at 3 μg/ml.

**LOX i.p. Tumor Model.** Male Swiss nu/nu nude mice (about 5 weeks old) were obtained from Taconic Farms, Inc. (Germantown, NY). Group housing (5/cage) was provided in polycarbonate cages with wire top and filters. Mice were allowed to acclimate for 1 week prior to experiments. Only normal, healthy mice were used. Human melanoma LOX cells used in this model were first grown s.c. in nude mice. On the day of i.p. implantation, tumors were excised, and a single-cell suspension was prepared. RBC were lysed by ammonium chloride. Each mouse received 2 X 10$^6$ LOX cells (trypan blue-negative) in 0.2 ml of PBS by i.p. injection. MKT-077 was administered i.p. for LOX tumor-bearing mice at 5 mg/kg on days 1–5.

**A498 and DU145 s.c. Tumor Models.** Human prostate carcinoma DU145 and human renal carcinoma A498 xenographs were propagated by s.c. serial transplantation in male (Taconic Farm) and female (Simonsen Laboratories, Inc., Gilroy, CA) Ncr-nu nude mice, respectively. On day 0, tumors (about 5 cm in diameter) were carefully trimmed to remove all connective tissues, cut into fragments of 4 mm$^3$, and implanted s.c. with a 12-gauge trocar needle. MKT-077 was administered i.v. for A498 tumor-bearing mice at 7.5 mg/kg on days 1, 3, 5, 7, 9, 11, and 13 and for DU145 tumor-bearing mice at 11.25 mg/kg on days 5, 7, 9, 11, 13, 15, 17, 23, and 25. Correspondingly, untreated tumor-bearing mice serving as controls received 5% dextrose on the same days as the treated mice. Tumors in excess of 1 g were produced in untreated controls within 30 days for both A498 and DU145.

**Laser Scanning Confocal Fluorescence Microscopy.** Mixed CV-1 and CV-1 cells grown on 12-mm glass coverslips were exposed to MKT-077 (3 μg/ml in culture medium) at 37°C for the indicated duration, washed, and mounted on a living cell chamber made of silicon rubber (15). MKT-077 fluorescence was detected by a Zeiss LSM inverted confocal microscope (excitation at 488 nm and emission at 543 nm). Digitized fluorescent images were stored in a microcomputer driven by Adobe Photoshop 3.1 (Adobe System, Inc.) and printed by a Fuji Pictography 3000 color printer (Fuji Photo Film USA, Inc., Elmsford, NY).

**Flow Cytometry.** CX-1 and CV-1 cells (10$^6$ in 25 ml of culture medium) were incubated with MKT-077 (3 μg/ml) at 37°C for 4 h. During incubation, the cell suspension was shaken gently, and after centrifugation, the cell pellet was resuspended in 1 ml of fresh medium. Fluorescent intensity was analyzed by a flow cytometer (Becton Dickinson FACScan) with excitation at 488 nm and emission at 525 nm.

**Extraction of MKT-077 from Cells for Measurement.** CX-1 cells (3 × 10$^7$) or CV-1 cells (5 × 10$^7$) in 50 ml of culture medium were treated with MKT-077 (3 μg/ml) at 37°C for the indicated time. During incubation, the cell suspension was shaken gently, and after centrifugation, the cell pellet was resuspended in PBS and recentrifuged. The pellet was then extracted with 3 ml of ethanol. After vortexing and further centrifugation, the supernatant was analyzed for the concentration of MKT-077 by a spectrophotometer.

**RESULTS**

**Chemical Structure of MKT-077.** Fig. 1 presents the structure of MKT-077 with chloride as a counter ion, a characteristic that greatly enhances its water solubility. MKT-077 is a typical lipophilic compound with a delocalized positive charge. Such an agent is known to enter cells by membrane potential-driven simple diffusion (1).

**Effects of MKT-077, Cisplatin, and Adriamycin in Clonogenic Assays.** Fig. 2a shows the effect of MKT-077 on the colony formation of the normal monkey kidney cell line CV-1 and five human tumor cell lines, including CX-1 (colon), MCF-7 (breast), CRL-1420 (pancreas), EJ (bladder), and LOX (melanoma), after 24-h treatments. IC$_{50}$s of MKT-077 are 0.15 μg/ml for LOX and CRL-1420, 0.25 μg/ml for CX-1, 0.30 μg/ml for EJ, 0.5 μg/ml for MCF-7, and 40 μg/ml for CV-1. For the most responsive cell lines, LOX and CRL-
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300
200
(3 μg/ml) was clearly evident. After 24 h treatment, the morphology of MCF-7 cells became abnormal and was accompanied by the cessation of normal ruffling activity. After 48 h, most cells underwent rapid blebbing, followed by cell burst. In contrast, under the same conditions, CV-1 cells grew rapidly, despite the daily addition of MKT-077.

Antitumor Activity in Nude Mice. Fig. 5 shows that after i.p. implantation of 2 × 10^6 LOX cells, male nude mice had a median survival of 18 days. In contrast, mice that received i.p. injections of MKT-077 (5 mg/kg; days 1–5) showed a median survival of 62 days with T/C = 344%. Two of the five MKT-077-treated mice in this experiment were still alive on day 80. Effects of MKT-077 in the LOX i.p. model were examined in an additional 35 trials by varying doses and schedules. Although efficacy varied, MKT-077 consistently displayed antitumor activity similar to that shown in Fig. 5. In one notable experiment in which MKT-077 was injected i.p. at 5 mg/kg on days 1, 5, 8, 11, and 14, the median survival observed was 97 days with T/C = 539%.

Fig. 6 shows the response of a human renal carcinoma A-498 s.c. xenograft to i.v. treatment with MKT-077. As indicated by the mean tumor size in both control and treated mice, MKT-077 (7.5 mg/kg i.v. on days 1, 3, 5, 7, 9, 11, and 13) exhibited significant antitumor activity up to 43 days after tumor implantation.

The response of human prostate carcinoma DU-145 s.c. xenografts to i.v. treatment with MKT-077 is shown in Fig. 7. MKT-077 administered i.v. at 11.25 mg/kg on days 5, 7, 9, 11, 15, 17, 23, and 25 showed marked inhibition of tumor growth.

MKT-077 Uptake Visualized by Confocal Microscopy. To understand the basis for the antitumor activity of MKT-077, confocal fluorescence microscopy was used to localize the fluorescence of MKT-077. Fig. 8 depicts a coculture of CRL-1420 and CV-1 cells incubated with MKT-077 at 3 μg/ml for 30 min and examined by confocal microscopy. MKT-077 fluorescence is localized selectively in the mitochondria of CRL-1420 cells. Under this condition, as a result of its low membrane potential (1–13, 16–24), the mitochondria of CV-1 failed to take up sufficient MKT-077 for visualization. It is possible to visualize CV-1 mitochondria at a much higher concentration of MKT-077 (e.g., 100 μg/ml for 30 min).

Fig. 3. Selectivity of MKT-077, cisplatin, and Adriamycin between CV-1 and five human cancer cell lines by clonogenic assays (CX-1, MCF-7, LOX, EJ, and CRL-1420).

Fig. 4. Effect of MKT-077 on the growth of CV-1 and human breast carcinoma MCF-7 in mass cultures. MKT-077 at 3 μg/ml was added daily for 4 days.

1420, MKT-077 is 267-fold more effective in inhibiting colony formation than it is for CV-1 cells. Fig. 2b shows the effect of cisplatin on the same cell lines under the same conditions. It appears that cisplatin does not display significant selectivity between CV-1 and five human tumor lines in these assays. Fig. 3 compares the selectivity of cisplatin, Adriamycin, and MKT-077 among five human tumor cell lines. Whereas MKT-077 displays high selectivity between CV-1 and human tumor lines, cisplatin does not.

Effect of MKT-077 on Growth Rate of MCF-7 and CV-1. Fig. 4 shows the effect of MKT-077 on the cell growth of MCF-7 and CV-1 in mass culture. MKT-077 at 3 μg/ml (daily replenishment) has no effect on the growth of CV-1 cells. In contrast, it has a marked effect on the growth of MCF-7 under the same culture conditions.

Time-Lapse Videomicroscopy. The effect of MKT-077 on cell growth was also confirmed by time-lapse videorecording in which the inhibition of MCF-7 cell division soon after the addition of MKT-077 (3 μg/ml) was clearly evident. After 24 h treatment, the morphology of MCF-7 cells became abnormal and was accompanied by the cessation of normal ruffling activity. After 48 h, most cells underwent rapid blebbing, followed by cell burst. In contrast, under the same conditions, CV-1 cells grew rapidly, despite the daily addition of MKT-077.

Fig. 5. Survival of human melanoma LOX-bearing nude mice treated with MKT-077 i.p. at 5 mg/kg on days 1–5. T/C = 344%.

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MKT-077 Uptake Monitored by Flow Cytometry. We used flow cytometry to compare the uptake of MKT-077 by CX-1 and CV-1 cells. After a 5-h treatment with MKT-077 at 3 μg/ml, CX-1 cells took up MKT-077 to a significantly higher extent than CV-1 cells (Fig. 9).

Quantitation of MKT-077 Uptake and Retention by Ethanol Extraction. The uptake of MKT-077 by CX-1 and CV-1 cells was also determined by ethanol extraction followed by spectrophotometric measurements. Fig. 10 shows that the uptake of MKT-077 after a 6-h incubation is 65-fold greater by CX-1 cells than by CV-1 cells. In addition, after MKT-077 was removed from the culture medium, CX-1 cells continued to retain a significant amount of MKT-077, even after 20 h in drug-free medium (Fig. 10). The small amount of MKT-077 taken up by CV-1 cells was rapidly released into the culture medium once cells were placed in drug-free medium.

DISCUSSION

The results of this study demonstrate that MKT-077 has significant growth-inhibitory activity against a variety of keratin-positive human cancer cell lines in culture, as measured by clonogenic assays and cell growth inhibition in mass cultures. MKT-077 also displays significant antitumor activity in nude mice implanted with human melanoma LOX, human renal carcinoma A498, and human prostate carcinoma DU145, all of which are highly refractory to various therapies.

Differences in the mitochondrial membrane potential between carcinoma cells and normal epithelial cells have been noted previously (1–7, 13, 16–27). Dairkee et al. (27) reported recently that primary breast epithelial cells derived directly from carcinoma explants display increased uptake and retention of rhodamine 123 when compared with epithelial cells derived from normal mammary explants. Earlier studies using normal primary bladder, breast, and kidney epithelial cells and a variety of epithelial cell lines derived from normal tissues have demonstrated that respective mitochondrial membrane potentials, as indicated by rhodamine 123 or tetraphenylphosphonium uptake, are very similar to that of CV-1 cells (1–13, 16–24). For convenience, we have used CV-1 as an indicator cell line for cells with low mitochondrial membrane potentials in this study. CV-1 cells used here do not express P-glycoprotein, as examined by immunofluorescence microscopy and immunoelectron microscopy. In contrast to cells expressing P-glycoprotein, which render them Adriamycin resistant, the CV-1 cells used here are highly sensitive to Adriamycin (Fig. 3).

Like other DLCs, MKT-077 is accumulated and retained by the mitochondria of carcinoma cells to a much greater extent than by normal epithelial cells. The mitochondrial membrane potentials of CV-1 and CX-1 cells have been estimated previously to be 104 and 163 mV, respectively (21). This 60-mV difference (equivalent to 120,000 V across 1 cm) may generate a significant difference in the concentration gradient. Based on the Nernst equation, a 60-mV potential difference would account for a 10-fold difference in the accumulation of MKT-077 (1, 3, 18, 28–32). Since the plasma membrane potential is also higher in carcinoma cells, a higher cytoplasmic concentration of MKT-077 in carcinoma cells relative to normal cells would result (18, 29, 30). Thus, the increased uptake of MKT-077 by carcinoma cells could be much higher than the 10-fold difference predicted by the mitochondrial membrane potentials alone. Indeed, Figs. 9 and 10 show that CX-1 cells could take up 20- to 65-fold more MKT-077 than CV-1 cells.

Thus, the simplest explanation for selective toxicity observed between CV-1 cells and carcinoma cells may be the differential uptake of MKT-077 by these cells. Since MKT-077 is accumulated in mitochondria, one possibility might be that the molecular targets of MKT-077 reside in mitochondria. Indeed, in the accompanying manuscripts, we have observed selective mitochondrial toxicity by MKT-077 in several different carcinoma cell lines at the cellular, biochemical, and molecular levels (33, 34).

However, a higher mitochondrial membrane potential in carcinoma cells and the resulting increased uptake of MKT-077 may not be sufficient for achieving selective cytotoxicity described here. If these were the case, cardiac muscle cells, which have high mitochondrial membrane potential (16), would be especially susceptible to MKT-077 toxicity. Significant cardiac toxicity, however, has not yet been

Fig. 6. Effects of MKT-077 on the growth of human renal carcinoma A-498 s.c. in nude mice. Twenty tumor-bearing mice were used as untreated controls. Ten mice were treated with MKT-077 at 7.5 mg i.v. on days 1, 3, 5, 7, 9, 11, and 13.

Fig. 7. Effects of MKT-077 on the growth of human prostate carcinoma DU145 s.c. in nude mice. Twenty tumor-bearing mice were used as untreated controls. Ten mice were treated with MKT-077 at 11.5 mg/kg i.v. on days 5, 7, 9, 11, 15, 17, 23, and 25.
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Fig. 8. Differential uptake of MKT-077 between CV-1 and human pancreatic carcinoma CRL-1420. Cells cocultured on coverslips were exposed to MKT-077 at 3 µg/ml for 30 min. *Left*, confocal fluorescent micrograph; *right*, phase-contrast of the same field.

Exert any myelotoxicity. Thirty patients with refractory carcinomas have been treated with MKT-077 in Phase I clinical trials by a 30-min infusion per day on days 1, 3, and 5, followed by a 3-week rest period. At a dose of 48 mg/m², MKT-077 continues to elicit no serious adverse effects, including cardiac toxicity and myelotoxicity in these patients.

There is an urgent need to develop novel selective and effective therapies for human carcinomas, such as those of the lung, breast, colon, prostate, and pancreas. MKT-077 may represent the first of a new class of antineoplastic agents, based on their mitochondrial accumulation and high degree of selectivity for carcinomas without major untoward effects on vital organs such as bone marrow. In this light, MKT-077 could also be useful in combination therapy for cancer, since mitochondria have never before been exploited as a target for cancer chemotherapy. Finally, because MKT-077 is accu-

detected in preclinical toxicology studies involving rodents and dogs. This implicates the involvement of additional cellular factor(s) unique to carcinoma cells in the manifestation of the overall cytotoxicity expressed by MKT-077. Conceivably, these factors could reside outside of mitochondria. Previously, we have shown that mitochondria can serve not only as a reservoir but also as a slow-release device for DLCs such as MKT-077. The cellular factors unique to carcinoma cells may be preferentially affected by MKT-077, which could account for the dramatic selectivity seen in the clonogenic assays.

It is of interest to note that in addition to the lack of cardiac toxicity, MKT-077 also fails to induce myelotoxicity, a serious side effect experienced by many conventional anticancer drugs. Although bone marrow cells proliferate very rapidly, their mitochondrial membrane potentials are low and little MKT-077 is accumulated. Preclinical toxicology studies in rats and dogs, as well as Phase I clinical trials, have borne out the expectation that DLCs such as MKT-077 do not

Fig. 9. MKT-077 differential uptake between CV-1 and human colon carcinoma CX-1 as monitored by flow cytometry.

Relative Fluorescence Intensity

Fig. 10. Uptake and retention of MKT-077 by CV-1 and human colon carcinoma CX-1. MKT-077 was extracted by ethanol and measured by absorption spectra.

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mulated in mitochondria where oxygen is most abundant, it might also serve as a sensitizer for hyperthermia, radiation, or photodynamic therapy.

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REFERENCES

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