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Preclinical study

Activity of MKT 077, a rhodacyanine dye, against human tumor colony-forming units

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MKT 077 is related to rhodamine 123 dye and demonstrates preferential accumulation in the mitochondria of cancer cells compared to normal cells. This difference in retention between cancer and normal cells led to the finding that MKT 077 selectively inhibits the growth of cancer cells *in vitro*. To define the preclinical activity profile of MKT 077, the compound was tested *in vivo* against a large variety of human tumors utilizing the human tumor-cloning assay. MKT 077 was studied using a sequential 2 h exposure separated by 24 h (2–24–2 h) and a 24 h exposure at final concentrations of 0.1, 0.2, 1.0, 2.0, 10.0 and 20.0 μg/ml. MKT 077 was also studied using continuous exposure at final concentrations of 0.1, 1.0 and 10 μg/ml. A decrease in tumor colony formation was considered significant if survival of colonies treated with MKT 077 was 50% or less compared to untreated controls. A total of149 specimens was treated with MKT 077 with 51, 58 and 34 evaluable specimens with the 2–24–2 h, the 24 h and the continuous exposure, respectively. The results of the present study suggest a positive relationship between concentration and response. No relationship between exposure schedule and activity was observed. Inhibitory effects were obtained against multiple tumor types. High cytotoxic activity was obtained against breast, ovary, endometrial, colon and non-small cell lung cancer with concentrations of 2 μg/ml or above. In conclusion, the broad spectrum of cytotoxicity of MKT 077 in the human tumor-cloning assay and the unique mechanism of action of MKT 077 encourage additional preclinical and clinical studies with this compound and other rhodacyanine dyes. [© 1999 Lippincott Williams & Wilkins.]

Key words: Human tumor cloning assay, MKT 077, rhodacyanine dyes.

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Introduction

Tumor supressor genes and amplified oncogenes are critical elements in the pathogenesis of cancer. However, cytoplasmic elements are also necessary in development and maintenance of the neoplastic phenotype. In fact, it was been demonstrated that cytoplasm from transformed cells could transfer the malignant phenotype to karyoplasts of normal cells.[1] One candidate organelle for cytoplasmic support of neoplastic transformation is the mitochondria. Mitochondria contribute to the cellular redox balance and the adenosine triphosphate pool required for cell cycle progression.[2] There are data to suggest that functional aspects of the mitochondria play a role in maintaining the tumorigenic phenotype.[3] That point was particularly demonstrated when the tumorigenic phenotype was altered after depletion of mitochondria in brain and breast tumor cells.[4] In human carcinoma cells, the mitochondrial membrane potential was shown to be increased compared to normal cells.[5,6] This elevation in the mitochondrial membrane potential is responsible for increased uptake and prolonged retention of lipophilic cations.[7,8] Due to its preferential accumulation in cancer cell mitochondria, lipophilic cations have been proposed as a potential novel class of chemotherapeutic agents.

Rhodacyanine dyes are lipophilic cations that have already demonstrated *in vitro* and *in vivo* anti-tumor activity.[9] MKT 077 (Figure 1) is related to rhodamine 123 dye, which is often used in mitochondria staining for fluorescent microscopy.[10] Rhodamine 123 demonstrated preferential accumulation in cancer cells compared to normal cells.[11] The mitochondria of a variety of carcinomas were shown to retain rhodamine 123 for days, whereas normal cells released it within a few hours.[6] This difference in rhodamine

123 retention between carcinoma and normal cells was exploited when it was demonstrated that this compound inhibits selectively the growth of carcinoma cells *in vitro*.[12] Compared to other members of this class, MKT 077 has two features that enhances its potential value as a chemotherapeutic agent. These features include enhanced stability in aqueous solutions and lesser light sensitivity than the other rhodacyanine compounds.[13] A previous study has demonstrated that MKT 077 had a greater than 250-fold more effective inhibiting activity on tumor cell lines than on normal cells.[13]

To additionally define the preclinical activity profile of MKT 077, the compound was tested against a large variety of human tumors utilizing the human tumor-cloning assay.[14] In a trial completed by the NCI, the cloning assay has shown the ability to distinguish clinically active from clinically inactive compounds.[15] In the present study, tumor specimens were taken both from patients who were untreated as well as those who had received prior therapy. These humans tumors were exposed to MKT 077 *in vitro* and the response (defined as 50% or less survival of colony-forming units compared to untreated controls) was evaluated by quantitating the formation of human tumor colony-forming units.

Material and methods

Drug

MKT 077, 1-ethyl-2-{[3-ethyl-5-(3-methylbenzothiazolin-2-yliden)]-4-oxothiazolidin-2-ylidenemethyl}pyridium chloride, was provided by Novartis (Basel, Switzerland). MKT 077 was dissolved in water to make a concentrated stock solution of 1 mg/ml. MKT 077 is very stable and can be stored in aqueous solution at 4 C for more than 1 year. Aliquots of 0.5 ml of the stock solution were stored at −70 C and were thawed prior to use for each new tumor sample tested. At the time of testing, aliquots of the stock solution were further diluted in water to make the desired final concentrations.

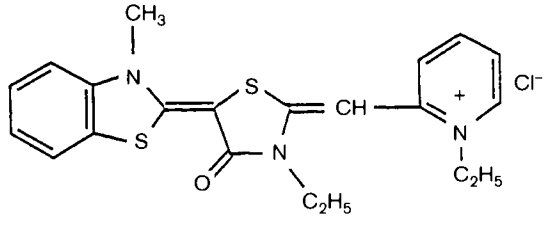

Figure 1. Chemical structure of MKT 077.

In vitro exposure

Three different schedules were studied: two sequential 2 h exposures separated by 24 h (2-24-2 h), 24 h exposure and continuous exposure (14 days). The sequential exposure was chosen because of sequential exposures used in clinical trials. At 2 and 24 h exposures, the final concentrations evaluated were 0.1, 0.2, 1.0, 2.0, 10.0 and 20.0 μg/ml. At continuous exposure (14 days), the final concentrations evaluated were 0.1, 1.0 and 10 μg/ml.

The tumor cells treated with MKT077 with the sequential 2 h exposure (2.0 μg/ml) and with the continuous exposure (1.0 μg/ml) were also exposed to standard anticancer agents for 1 h at the following concentrations (μg/ml): doxorubicin, 0.04; cisplatin, 0.2; paclitaxel, 2.5. The single concentration of each drug corresponded to approximatively 1/10 of the peak plasma concentration for each drug in humans[16] and was predictive of clinical response in previously published studies.[17]

Human tumor colony-forming assay

After obtaining informed consent in accordance with federal and institutional guidelines, malignant effusions, ascites, bone marrow aspirates containing tumor cells as well as solid tumor specimens were collected from patients undergoing tissue procurement procedures as part of their diagnostic work-up or as part of their routine therapeutic care. Solid tumors or malignant lymph nodes were minced into 2-5 mm fragments in the operating room and immediately placed in McCoys medium 5A (Life Technologies, Grand Island, NY) plus 10% heat-inactivated newborn calf serum and 1% penicillin/streptomycin. Within 4 h, these solid tumors were mechanically dissociated with scissors, forced through a no. 100 stainless steel mesh, through 25 gauge needles and then washed with McCoys medium. Ascitic, pleural, pericardial fluids and bone marrow aspirates were obtained by standard techniques. The fluid or marrow was placed in sterile containers containing 10 U of preservative-free heparin (ONeill, Johns and Feldman, St Louis, MO)/ml of malignant fluid or marrow. After centrifugation at 150 g for 10 min, the cells were harvested and washed with McCoys medium plus 10% heat-inactivated fetal calf serum. The viability of cell suspensions was determined on a hemocytometer with Trypan blue.

Only viable cells determined the final concentration of plated cells.

In vitro exposure of tumor cells to the drug

For the 2-24-2 h and 24 h exposure studies, the cells were incubated for 2 and 24 h, respectively, with MKT 077 in McCoys medium, then washed to stimulate the disappearance of the drug from the body. MKT 077 was reintroduced 24 h after, during 2 h for the 2-24-2 h exposure. Cells were suspended in 0.3% agar in enriched Connaught Medical Research Laboratories (CMRL) medium 1066 (Irvine Scientific, Irvine, CA) supplemented with 15% heat-inactivated horse serum, penicillin (100 U/ml), streptomycin (2 mg/ml), glutamine (2 mmol/l), insulin (3 U/ml), asparagine (0.6 mg/ml) and HEPES buffer (2 mmol/l). Cells were plated in 35 mm Petri dishes in a top layer of agar over an underlayer of 0.3% agar to prevent growth of fibroblasts. Three plates were prepared for each data point. For the continuous exposure, cells were combined with MKT 077 in the CMRL medium described above, then plated as for the 2 h and 24 h exposure. The compound was not washed from the culture during the 2 week period and additional drug was not added to the culture. The plates were placed in a 37 C incubator and removed on day 14 for colony count. The number of colonies, defined as more than 50 cells, formed on the three drug-treated plates was compared to the number of colonies formed on the three untreated control plates and the percent colonies surviving at each concentration was calculated. An evaluable test is defined as one averaging at least 20 colonies on day 14 on the untreated control plates.

Quality control

To assure the presence of a single-cell suspension on the day of plating, positive controls were used to assess the effect on colony formation. For each tumor tissue sample tested, three positive control plates were set up to contain the non-specific cellular toxin orthosodium vanadate at a concentration of 200 μg/ml, which completely inhibits colony growth. For a tumor sample test to be considered evaluable, orthosodium vanadate had to produce less than 30% survival of colony-forming units when compared to untreated control plates. When survival was greater than 30%, indicating that single-cell suspension on day 0 was poor since orthosodium vanadate does not affect clumps, the tumor sample test was considered non-evaluable. The use of a positive control has been demonstrated to greatly enhance the reproducibility of the human tumor-cloning assay.

A test is defined as an experiment, performed on a unique tumor tissue sample, that contains untreated control, positive control and three or four specified drug concentration levels.

Data analysis and statistics

The results were expressed as the percentage of survival of tumor colony-forming units for a particular drug relative to its control. This quantity was calculated as the ratio of the average number of colonies surviving on the three drug-treated plates to the average number of colonies growing on the three untreated control plates. A ratio of 0.5 or less denotes a positive response, suggesting a significant inhibition of colony formation by the drug. Statistical comparisons of the response rates were performed using the McNemar test with two-sided p values. $p < 0.05$ was considered to indicate statistical significance.

Results

Evaluable specimens

A total of149 specimens was obtained from cancer patients. One hundred and twenty-four specimens were treated with MKT 077 at concentrations of 0.1, 0.2, 1.0, 2.0, 10.0 and 20.0 μg/ml using the 2-24-2 h exposures. Among these specimens, 41% (51 of 124) were evaluable with appropriate positive controls (Table 1). MKT 077 at the same concentations was also tested using a 24 h exposure on 142 specimens. Among these specimens, 41% (58 of 142) were evaluable with appropriate positive controls (Table 2). A continuous exposure with MKT 077 at concentrations of 0.1, 1.0 and 10.0 μg/ml was tested on 93 specimens. Among these specimens, 36% (34 of 93) were evaluable with appropriate positive controls (Table 3).

In vitro responses

When MKT 077 was evaluated using a 2-24-2 h exposure (Table 1), *in vitro* responses (50% or lower survival compared to untreated controls) were observed in 9% (three of 32), 19% (three of 16), 34% (11 of 32), 50% (nine of 18), 57% (four of seven) and 100% (six of six) at concentrations of 0.1, 0.2, 1.0, 2.0, 10.0 and 20.0 μg/ml, respectively. The *in vitro* response

rate at 0.2 μg/ml was significantly greater than 0.1 μg/ml ($p<0.001$). There was no significant difference in the responses rates between 0.2 and 1.0 μg/ml ($p=0.44$), 1.0 and 2.0 μg/ml ($p=0.26$), and 0.2 and 2.0 μg/ml ($p=0.23$). The *in vitro* response rate with 10 μg/ml was significantly greater than with 0.2 μg/ml ($p=0.013$).

With an exposure to MKT 077 for 24 h (Table 2), *in vitro* responses were observed in 6% (two of 31), 15% (two of 13), 48% (15 of 31), 68% (19 of 28), 86% (six of

Table 1. Number of sensitive specimens among evaluable specimens according to the concentration of MKT 077 with 2–24–2 h exposure, sensitivity being defined as 50% or less survival at the given concentration

Tumor type	Concentration of MKT 077 (μg/ml)					
	0.1	0.2	1.0	2.0	10.0	20.0
Breast	0/4	0/1	2/4	0/1	–	1/1
Colon	0/2	0/1	1/2	1/2	–	1/1
Kidney	0/3	1/1	0/3	1/1	1/1	1/1
Hepatoma	0/1	–	1/1	–	–	–
Lung—non-small cell	1/9	0/1	3/9	0/1	0/2	–
Lung—small cell	0/1	–	0/1	–	–	–
Melanoma	–	0/2	–	2/2	–	–
Mesothelioma	–	0/1	–	0/1	–	–
Ovary	0/3	1/5	1/3	2/5	1/2	1/1
Pancreas	–	0/1	–	0/1	–	–
Peritoneum cancer	–	1/1	–	1/1	–	1/1
Prostate	0/4	–	1/4	–	–	–
Sarcoma	0/1	–	0/1	–	–	–
Thyroid	0/1	–	0/1	–	–	–
Endometrium	2/2	–	2/2	–	2/2	–
Unknown primary carcinoma	0/1	0/2	0/1	2/3	–	1/1
Total	3/32	3/16	11/32	9/18	4/7	6/6

–, not tested.

Table 2. Number of sensitive specimens among evaluable specimens according to the concentration of MKT 077 with 24 h exposure, sensitivity being defined as 50% or less survival at the given concentration

Tumor type	Concentration of MKT 077 (μg/ml)					
	0.1	0.2	1.0	2.0	10.0	20.0
Breast	1/4	0/1	2/4	2/3	–	2/3
Colon	0/2	0/1	2/2	1/2	–	1/1
Kidney	0/3	–	1/3	1/1	1/1	1/1
Hepatoma	0/1	–	1/1	–	–	–
Lung—non-small cell	0/8	0/1	5/8	3/3	1/1	2/2
Lung—small cell	0/1	–	1/1	–	–	–
Melanoma	–	0/2	–	2/3	–	1/1
Mesothelioma	–	0/1	–	1/1	–	–
Ovary	1/4	1/5	1/4	6/9	2/3	4/4
Pancreas	–	–	–	0/1	–	–
Peritoneum cancer	–	0/1	–	1/1	–	1/1
Prostate	0/4	–	1/4	–	–	–
Sarcoma	0/1	–	0/1	–	–	–
Stomach	–	–	–	0/1	–	0/1
Thyroid	0/1	–	1/1	–	–	–
Endometrium	0/2	–	0/2	–	2/2	–
Unknown primary carcinoma	–	1/1	–	2/3	–	2/2
Total	2/31	2/13	15/31	19/28	6/7	14/16

–, not tested.

Table 3. Number of sensitive specimens among evaluable specimens according to the concentration of MKT 077 with continuous exposure, sensitivity being defined as 50% or less survival at the given concentration

Tumor type	Concentration of MKT 077 (μg/ml)		
	0.1	1.0	10.0
Breast	0/1	1/2	1/1
Colon	0/1	1/2	1/1
Kidney	0/2	1/2	–
Lung—non-small cell	0/3	2/5	1/2
Melanoma	0/2	2/2	–
Mesothelioma	0/1	0/1	–
Ovary	0/8	3/12	2/4
Pancreas	0/1	1/1	–
Peritoneum cancer	0/1	0/1	–
Sarcoma	1/1	0/1	–
Stomach	–	0/1	1/1
Endometrium	0/1	1/1	–
Unknown primary carcinoma	1/1	3/3	2/2
Total	2/23	15/34	8/11

–, not tested.

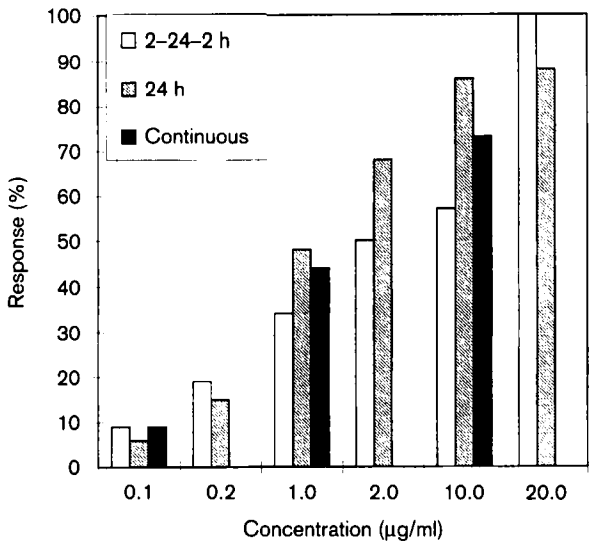

Figure 2. Relationship between concentration of MKT 077 and response.

seven) and 88% (14 of 16) at concentrations of 0.1, 0.2, 1.0, 2.0, 10.0 and 20.0 μg/ml, respectively. The *in vitro* response rate was significantly greater for 0.2 compared to 0.1 μg/ml ($p<0.001$) and for 1.0 compared to 0.2 μg/ml ($p<0.001$). There was no significant difference in the *in vitro* responses rates between 1.0 and 2.0 μg/ml ($p=0.11$).

In the study in which continuous exposure to MKT 077 was evaluated (Table 3), *in vitro* responses were noted in 9% (two of 23), 44% (15 of 34) and 73% (eight of 11) of specimens at concentrations of 0.1, 1.0 and 10.0 μg/ml, respectively. There was no significant difference in the responses rates between 0.1 and 1.0 μg/ml ($p=0.25$), and 1.0 and 10.0 μg/ml ($p=0.55$), but the *in vitro* response rate with 10 μg/ml was significantly greater than with 0.1 μg/ml ($p=0.012$).

The above results suggest that there may be a positive relationship between concentration and response but no trend favoring a particular schedule was observed (Figure 2). Indeed, the *in vitro* response rates were very similar with the same concentration with the different exposure schedules. Inhibitory effects were obtained against multiple tumor types, indicating that MKT 077 is a potent cytotoxic compound with a broad spectrum of antitumor activity. Of particular interest was potent cytotoxic activity noted against melanoma and renal tumors, when the concentration was 2.0 μg/ml or higher, whatever the exposure schedule. MKT 077 also has impressive activity against endometrial and ovary tumor colony-forming units.

Effects in tumors resistant to conventional chemotherapy

Among the 18 specimens tested at the concentration of 2.0 μg/ml with the sequential 2 h exposure, SCH 66336 had activity in six of 10 (60%) tumor specimens resistant to cisplatin and in five of seven (71%) tumor specimens resistant to paclitaxel (Table 4).

Among the 34 specimens tested at the concentration of 1.0 μg/ml with the continuous exposure, SCH 66336 had activity in two of five (40%) tumor specimens that were resistant to doxorubicin, in seven of 18 (39%) tumor specimens resistant to cisplatin and six of 16 (37%) tumor specimens resistant to paclitaxel (Table 4).

Discussion

In the present study using fresh tumors cultured in the human tumor-cloning assay, MKT 077 demonstrated prominent activity against various tumor types, including melanoma, breast, ovarian, endometrial, colon and renal tumor colony-forming units. In addition, potent cytotoxic activity was noted against hepatic, pleural and non-small cell lung tumors. There appeared to be a direct relationship between concentration and response (Figure 2). Contrary to a previous study,[18] the evaluation of MKT 077 in the present study did not demonstrate a relationship between the cytotoxic activity and the duration of exposure,

Table 4. Comparison of MKT 077 with other agents

Agent	MKT 077 2.0 μg/ml 2–24–2 h exposure					MKT 077 1.0 μg/ml continuous infusion				
	S/S	S/R	R/S	R/R	Total	S/S	S/R	R/S	R/R	Total
Doxorubicin	–	1	1	–	2	1	2	2	3	8
Cisplatin	2	6	–	4	12	2	7	3	11	23
Paclitaxel	1	5	2	2	10	2	6	3	10	21

S/S, sensitive to both MKT 077 and the agent;
S/R, sensitive to MKT 077 but resistant to the agent;
R/S, resistant to MKT 077 but sensitive to the agent;
R/R, resistant to both MKT 077 and the agent.
Sensitive defined as 50% or less survival at the given concentration.
–, not tested.

suggesting a threshold effect. The prolonged retention of MKT 077 in tumor cell mitochondria might explain why the extended duration of exposure did not increase the response rate. This cytotoxic activity in our tumor-cloning assay was obtained with MKT 077 concentrations which are similar to mean peak values of MKT 077 plasma concentrations in phase I study (1.1–2.8 μg/ml).[19]

Like others lipophilic cations, MKT 077 is accumulated and retained by the mitochondria of malignant cells to a much greater extent than by normal cells.[13] The increased uptake of MKT 077 by human breast cancer cells has been shown to be 20- to 65-fold more than in normal cells.[13] To explain the cytotoxicity of MKT 077, evidence was found for inhibition of electron transport through mitochondrial membranes by this compound and inhibition of mitochondrial respiration.[20] MKT 077-treated tumor cells have also demonstrated a selective loss of mitochondrial DNA.[20] Previous reports have shown that MKT 077 has potent growth-inhibitory activity against five human cancer cell lines (colon, breast, pancreas, bladder cancer and melanoma).[13] The IC_{50} for these different human tumor cell lines was between 0.15 and 0.50 μg/ml.[13] It was also demonstrated that MKT 077 displays significant antitumor activity in athymic mice implanted with human melanoma, renal carcinoma and prostate carcinoma.[13] Interestingly, little MKT 077 is accumulated in bone marrow cells.[21] Indeed, the mitochondrial membrane potentials of bone marrow cells is very low,[21] explaining the low myelotoxicity observed in preclinical studies and preliminary results of phase I studies with MKT 077.[13,19]

While lipophilic cations such as MKT 077 may constitute a potent class of anticarcinoma agents, they may be also used as carriers for drugs with no positive charge or as photodynamic sensitizers. A complex between rhodamine 123 and cisplatin was studied, and demonstrated to be taken up more by tumor cells than was cisplatin alone.[22] This association between rhodacyanine dye and cisplatin might have a potent synergistic association because cisplatin can display preferential binding to mitochondrial DNA versus genomic DNA in some cell lines.[23,24] Rhodacyanine dyes are well known as photosensitizers in the photography industry and numerous lipophilic cations have been reported to be photodynamic sensitizers.[25] The inhibition of mitochondrial function by MKT 077 can be enhanced 6-fold by photoactivation.[26] The damaging effect of MKT 077 on mitochondrial DNA was also increased by photoirradiation.[26] This possibility of localized irradiation of the tumor following selective accumulation of MKT 077 in the mitochondria of malignant offers a dual mechanism for treatment.

In summary, MKT 077 has demonstrated potent *in vitro* cytotoxic activity against a broad spectrum of human tumors in the human tumor-cloning assay. This drug is the first compound of the rhodacyanine dyes family in preclinical and clinical studies,[13,19] which has a unique mechanism of action among all the anticancer drugs, based on preferential accumulation in the mitochondria of malignant cells. The broad spectrum of cytotoxicity of MKT 077 in the human tumor-cloning assay and the unique mechanism of action of MKT 077 encourage additional preclinical and clinical studies with MKT 077 and rhodacyanine dyes.

References

1. Israel BA, Schaeffer WI. Cytoplasmic mediation of malignancy. *In Vitro Cell Dev Biol* 1988; **24**: 487–90.
2. Doward A, Sweet S, Moorehead R, Singh G. Mitochondrial contributions to cancer cell physiology: redox balance, cell cycle, and drug resistance. *J Bioenerg Biomembr* 1997; **29**: 385–92.

3. Hayashi JI, Takemitsu M, Nonaka I. Recovery of the missing tumorigenicity in mitochondrial DNA-less HeLa cells by introduction of mitochondrial DNA from normal human cells. *Somat Cell Mol Genet* 1992; **18**: 123-9.
4. Cavalli LR, Varella-Garcia M, Liang BC. Diminished tumorigenic phenotype after depletion of mitochondrial DNA. *Cell Growth Different* 1997; **8**: 1189-98.
5. Bernal SD, Lampidis TJ, McIssac RM, Chen LB. Anticarcinoma activity *in vivo* of rhodamine 123, a mitochondrial-specific dye. *Science*1983; **222**: 169-72.
6. Summerhayes IC, Lampidis TJ, Bernal SD, *et al.* Unusual retention of rhodamine 123 by mitochondria in muscle and carcinoma cells. *Proc Natl Acad Sci USA* 1982; **79**: 5292-6.
7. Davis S, Weiss MJ, Wong JR, *et al.* Mitochondrial and plasma membrane potentials cause unusual accumulation and retention of rhodamine 123 by human breast adenocarcinoma-derived MCF-7 cells. *J Biol Chem* 1985; **260**: 13844-50.
8. Nadakavukaren KK, Nadakavukaren JJ, Chen LB. Increased rhodamine 123 uptake by carcinoma cells. *Cancer Res* 1986; **45**: 6093-9.
9. Sun X, Wong JR, Song K, Hu J, Garlid KD, Chen LB. AA1, a new synthesized monovalent lipophilic cation, expresses potent *in vivo* antitumor activity. *Cancer Res* 1994; **54**: 1465-71.
10. Johnson LV, Walsh ML, Chen LB. Localization of mitochondria in living cells with rhodamine 123. *Proc Natl Acad Sci USA* 1980; **77**: 990-4.
11. Lampidis, TJ, Bernal SD, Summerhayes IC, Chen LB. Selective toxicity of rhodamine 123 in carcinoma cells *in vitro*. *Cancer Res* 1983; **43**: 716-20.
12. Bernal SD, Lampidis TJ, Summerhayes IC, Chen LB. Rhodamine-123 selectivity reduces clonal growth of carcinoma cells *in vitro*. *Science* 1982; **218**: 1117-9.
13. Koya K, Li Y, Wang H, *et al.* MKT-077, a novel rhodacyanine dye in clinical trials, exhibits anticarcinoma activity in preclinical studies based on selective mitochondrial accumulation. *Cancer Res* 1996; **56**: 538-43.
14. Hamburger AW, Salmon SE. Primary bioassay of human myeloma stem cell. *Science* 1977; **197**: 471.
15. Schoemaker RH, Wolpert-Defillipes MK, Kern DH, *et al.* Application of a human tumor colony-forming assay to new drug screening. *Cancer Res* 1985; **45**: 2134-53.
16. Alberts D, Chen H. Tabular summary of pharmacokinetic parameters relevant to *in vitro* drug assay. In: Salmon SE, ed. *Human tumor stem cells*. New York: Alan R Liss 1980: 351-9.
17. Von Hoff DD. Human tumor stem cell assay. *Curr Ther Hematol-Oncol* 1987; **3**: 365-7.
18. Kubota T, Chiba Y, Ishizuka H, *et al.* Antitumor activity of MKT-077, a delocalized lipophilic cation against human tumor cells *in vitro*. *Proc Am Ass Cancer Res* 1997; **38**: 217.
19. Britten CD, Rowinsky EK, Baker SD, *et al.* A phase I and pharmacokinetic study of the rhodocyanine dye analog MKT-077. *Proc Am Soc Clin Oncol* 1998; **17**: 231.
20. Modica-Napolitano JS, Koya K, Weisberg E, Brunelli BT, Li Y, Chen LB. Selective damage to carcinoma mitochondria by the rhodacyanine MKT-077. *Cancer Res* 1996; **56**: 544-50.
21. Chen LB. Mitochondria as a reservoir for anticancer drugs. *Anti-Cancer Drugs* 1989; **191**: 21-8.
22. Teicher BA, Holden SA, Jacobs JL. Intracellular distribution of a platinum-rhodamine 123 complex in *cis*-platinum sensitive and resistant human squamous carcinoma cell lines. *J Biol Chem* 1986; **35**: 3365-9.
23. Olivero OA, Semino C, Kassim A, Lopez-Larraza DM, Poirier MC. Preferencial binding of cisplatin to mitochondrial DNA of chinese hamster ovary cells. *Mutat Res* 1995; **346**: 221-30.
24. Daoud SS, Clements MK, Small CL. Polymerase chain reaction analysis of cisplatin-induced mitochondrial DNA damage in human ovarian carcinoma cells. *Anti-Cancer Drugs* 1995; **6**: 405-12.
25. Modica-Napolitano JS, Joyal JL, Ara G, Oseroff AR, Aprille JR. Mitochondrial toxicity of cationic photosensitizers for photochemotherapy. *Cancer Res* 1990; **50**: 7876-81.
26. Modica-Napolitano JS, Brunelli BT, Koya K, Chen LB. Photoactivation enhances the mitochondrial toxicity of the cationic rhodocyanine MKT-077. *Cancer Res* 1998; **58**: 71-5.

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