Antitumor Efficacy *in Vitro* and *in Vivo* of Falconensones, a New Type of Polyene[1]

Kayoko Tamagawa, Kazuyuki Shimizu, Takuya Ebine, Yoshie Maitani, Tetsuya Fukui, Ken-ichi Kawai, and Noriko Takahashi[2]

Faculty of Pharmaceutical Sciences, Hoshi University, Tokyo 142-8501, Japan

ABSTRACT

Falconensones A and B are new type of yellow pigment isolated from the mycelial extract of ascomycetous fungi, *Emericella falconensis*. To date, these falconensones and their derivatives, falconensone A *p*-bromophenylhydrazone and falconensone A dioxime are known to exhibit biological activities, which include growth inhibition and both induction of differentiation and apoptosis of HL60 human leukemia cells. The synthetic derivatives have been shown to be more potent than natural falconensone A and B in eliciting these activities. Herein, we investigate whether falconensones inhibit growth of other cancer cell lines *in vitro*, and we evaluate their ability to modify survival in C57 BL/6J mice using M5076 murine reticulosarcoma *in vivo*, which is established as the metastasis model. Falconensone A, falconensone A *p*-bromophenylhydrazone, and falconensone A dioxime inhibit growth of human myeloid leukemia cell lines, HL60 and HL60R, human hepatoma cell line HepG2, human prostate cancer cell line DU-145, and human breast cancer cell line MCF-7/Adr^R, whereas falconensone B, the 4′-nor-methyl derivative of falconensone A, shows extremely low or no activity. In contrast, all of the falconensones are active in growth inhibition of human breast cancer cell line MCF-7. Survival time of M5076-implanted mice was prolonged by treatment with falconensones, particularly falconensone A dioxime. These results indicate that falconensone A and its derivatives exhibit anticancer efficacy in a broad spectrum of cancer cell lines. These agents may have great potential for clinical use in the treatment of various cancers.

INTRODUCTION

Falconensone A and B are two new cyclopentenone derivatives isolated from mycelial extracts of ascomycetous fungi, *E. falconensis* (1). As shown in Fig. 1, the structures of these compounds are novel in containing cyclopent-2-enone rings connected at the ring 3 position to extensively conjugated methyl ketones. Falconensone B is the 4′-nor-methyl derivative of falconensone A. Two derivatives of falconensone A, falconensone A *p*-bromophenylhydrazone, which has a bromophenyl residue, and falconensone A dioxime, which possesses a hydroxy residue, were also synthesized (Fig. 1). These compounds were designed so as to incorporate features of RA,[3] including five ethylene units, a cyclohexene ring, and a carboxic acid, as well as a strongly ionized (active) residue (2).

Previous studies have shown that falconensones induce differentiation of HL60 human acute myeloid leukemia cells into monocyte/macrophage-like cells and that falconensone A enhances differentiation induced by RA in a synergistic manner (2). These results indicate that falconensone A influences the actions of RA. In addition, falconensone A derivatives and RA both exhibit antioxidant activity (3–5).

Falconensone A *p*-bromophenylhydrazone has a bromophenyl residue and is structurally related to fenretinide, 4-HPR having a hydroxyphenyl residue (Fig. 1). 4-HPR is a RA derivative, which is an effective chemopreventive (6) and antiproliferative agent (7–9) against a wide variety of tumor types including breast, prostate, ovary, and bladder. 4-HPR is in clinical trials for malignancies of breast (10) and the bladder (11). 4-HPR and falconensone A *p*-bromophenylhydrazone alone are weak inducers of differentiation of HL60 cells as compared with RA (2, 12). 4-HPR and falconensone A *p*-bromophenylhydrazone are potent inducers of apoptosis in HL60 cells (13, 14). Various chemicals or factors which induce (or inhibit) apoptosis may be useful as anticancer agents (15).

In the process of tumor metastasis, primary neoplasms spread to different sites (organs and lymph nodes) in the body and resume growth. Malignant neoplasms vary in the expression of biological and metastatic properties. There are several models for studying cancer metastasis. Hepatic metastasis can be established in syngeneic C57 BL/6J mice by i.v. injection of murine reticulosarcoma (M5076; Refs. 16, 17). M5076 cells originate from ovary and metastasize preferentially to peritoneal viscera, particularly liver after i.v. injection. This cell line provides a good model for site-specific metastasis.

Although we have recently shown that falconensones, except falconensone B, exhibit anticancer effects *in vitro*, *e.g.,*
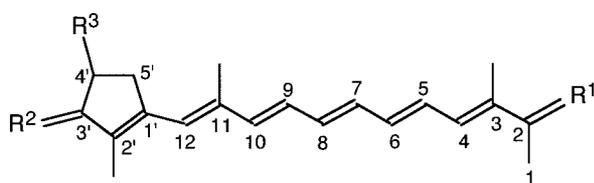
· falconensone A : R^1=O, R^2=O, R^3=CH$_3$

· falconensone B : R^1=O, R^2=O, R^3=H

· falconensone A *p*-bromophenylhydrazone :

R^1=NNH—〈 〉—Br, R^2=O, R^3=CH$_3$
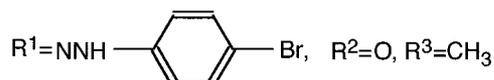
· falconensone A dioxime : R^1=N-OH, R^2=N-OH, R^3=CH$_3$

Fig. 1 Chemical structures of falconensones A and B, falconensone A *p*-bromophenylhydrazone, and falconensone A dioxime.

inhibition of growth and induction of differentiation and apoptosis of HL60 cells (2, 13), their effects on other cancer cell lines *in vitro* and cancers *in vivo* were not known. As reported herein, we find that falconensones inhibit the growth of various human cancer cell lines. These agents also significantly prolong survival times of mice with liver metastasis from implanted M5076 cells.

MATERIALS AND METHODS

Chemicals. Falconensones A and B were isolated from the mycelium of *E. falconensis*. Their structures have been determined by spectroscopic and X-ray analysis (1). Falconensone A *p*-bromophenylhydrazone and falconensone A dioxime were synthesized as described previously (1, 2, 18). RA and Ch were obtained from Sigma Chemical Co., St. Louis, MO. DPPC was purchased from NOF (Tokyo, Japan). SG containing β-sitosterol 3-β-D-glucoside, campesterol, stigmasterol, and brassicasterol was provided by Ryukakusan Co. (Tokyo, Japan). All of the other chemicals were of reagent grade.

Cells. Early passage (<30) human myeloid leukemia cell lines, HL60 (19) and HL60R, a mutant subclone of HL-60 that exhibits relative resistance to RA and that harbors RA receptors with markedly reduced affinity for RA (20, 21), were maintained in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 10 mM HEPES (pH 7.3) and 10% (v/v) FBS (Life Technologies, Inc.).

Human breast cancer cell lines MCF-7 and MCF-7/Adr^R (22) were obtained from the American Type Culture Collection, Rockville, MD. Cells were maintained in Iscove's modified Dulbecco's medium supplemented with 10% heat-inactivated FBS (Life Technologies, Inc.). Human hepatoma cell line HepG2 (23) was obtained from RIKEN cell bank (Tokyo, Japan). Human prostate cancer cell line DU-145 (24) was obtained from Dr. Yves G. Pommier of the National Cancer Institute (Bethesda, MD). HepG2 and DU-145 cells were grown in RPMI

1640 containing 10% FBS and subcultured every week. Attached cells were removed from the tissue culture flask surface with trypsin-EDTA (0.05% trypsin and 0.53 mM EDTA in HBSS without Ca^{2+} or Mg^{2+}; Life Technologies, Inc.).

Murine reticulosarcoma cell line (M5076) was obtained from Dr. Takao Yamori of the Cancer Chemotherapy Center, Japan Foundation for Cancer Research (Tokyo, Japan) and was grown in RPMI 1640 supplemented with 15% FBS as described previously (25).

All of the cells described above were incubated at 37°C in a humidified atmosphere of 5% CO$_2$ in air. Cell number was estimated using on an electric particle counter (Coulter Electronics, Hialeah, FL), and viability was determined by trypan blue dye exclusion.

Cell Growth. HL60 and HL60R or M5076 cells (2 × 10^5/ml) were grown in RPMI 1640 containing 10% FBS or 15% FBS, respectively, and various concentrations of compounds. M5076 cells were harvested by trypsinizing to dislodge cells into medium. Cell number was estimated by an electric particle counter and viability by trypan blue dye exclusion. The percentage of net growth is shown with values adjusted by subtracting the initial cell concentration of experimental cultures from the initial concentrations of control cultures, which were defined as 100% (Fig. 2). Values for percentage of net growth were calculated with the following formula: [(cell concentration of experimental culture) − (initial cell concentration)/(cell concentration of control culture) − (initial cell concentration)] × 100.

MCF-7, MCF-7/Adr^R, HepG2, and DU-145 cells were trypsinized and suspended in RPMI 1640 containing 10% FBS. Cells (1 × 10^4/ml) were incubated at 37°C in a humidified atmosphere of 5% CO$_2$ in air. After 1 day, various concentrations of compounds were added to the cultures. Cells were incubated for 72 h, and then viable cell number was estimated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide as described previously (26, 27). Values for percentage of net growth were calculated with the following formula: [(absorbance of experimental cell concentration) − (absorbance of initial cell concentration)/(absorbance of control cell concentration) − (absorbance of initial cell concentration)] × 100.

Preparation of Liposome-entrapped Compounds. Liposome-entrapped compounds were prepared from 60 μmol DPPC, 10 μmol SG, 30 μmol Ch, and 3 μmol compound (DPPC:SG:Ch:compound = 6:1:3:0.3 molar ratio) according to the reverse-phase evaporation method described previously (28–30). Liposome was stored at 4°C in the absence of light.

Quantitation of Compounds by High Pressure Liquid Chromatography. Compounds entrapped in liposomes were extracted with equivalent volumes of ethyl acetoacetate containing 1% acetic acid. After voltexing, mixtures were centrifuged at 10,000 × *g* for 5 min. Compound in organic layers were analyzed by high pressure liquid chromatography using a Shimazu LC-10A high pressure pump, Shimazu SIL-10A manual injector, and Shimazu RF-10AXL fluorescence detector (Shimadzu Co., Kyoto, Japan). A ZORBAX ODS column (C18 reverse-phase, 4.6 mm × 25 cm, 5 μm; Hewlett Packard, Palo Alto, CA) was used to separate RA and falconensones. The column was eluted with 10 mM of NH$_4$OAc in a methanol/H$_2$O gradient [70–90% (0–45 min), 90–100% (45–50 min), and then 100% (50–60 min)] at a flow rate of 1.5 ml/min with UV

Fig. 2 Growth of HL60 and HL60R cells in the presence of falconensones. Cells were grown without (◇) or with 1 μM RA (□) and falconensones: falconensone A (○), falconensone B (◉), falconensone A *p*-bromophenylhydrazone (△), and falconensone A dioxime (▲) at a concentration of 10 μM in medium containing 10% FBS. Measurements were made as described in "Materials and Methods." Each *point* is the mean of at least three measurements. The SE of each data point was ≤8% of the mean.
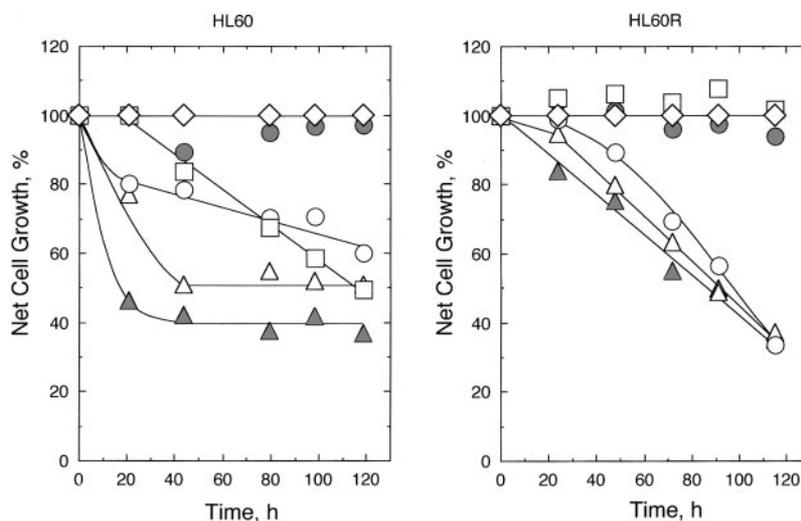
monitoring at wavelengths of maximum absorbance in each compounds, 408 nm for falconensone A, 440 nm for falconensone A *p*-bromophenylhydrazone, or 408 nm for falconensone A dioxime. Elution time was 17 min for falconensone A, 52 min for falconensone A *p*-bromophenylhydrazone, or 25 min for falconensone A dioxime. Measurements were made using the ratio of peak areas to internal standards.

Animals and Tumor Models. Specific pathogen-free female C57BL/6 mice (19–20 g, 7 weeks old) were purchased from Tokyo Laboratory Animal Science Company Co. Ltd. (Tokyo, Japan). M5076 cells were kept *in vivo* as s.c. solid tumors in C57BL/6 mice by transplantation every 2 weeks into the right axillary s.c. tissue. To obtain a suspension of tumor cells for transplantation, M5076 cells were prepared using 3 ml of sterile saline/g of s.c. solid tumor (3×10^6 cells/ml) and subsequently homogenized and suspended. This tumor cell suspension was injected i.v. into mice as described below.

Evaluation of Antitumor Activity. In the therapeutic experiments of liver metastatic cancer, a group of nine C57BL/6 mice were inoculated i.v. on day 0 with M5076 cells (6×10^5 cells, 0.2 ml) per mouse. On day 8 after implantation of tumor cells, mice were given compounds entrapped in liposomes as a single i.v. dose (0.6 mg/kg for falconensone A; 0.58 mg/kg for falconensone B; 0.93 mg/kg for falconensone A *p*-bromophenylhydrazone; and 0.66 mg/kg for falconensone A dioxime). Final blood concentration of each compound was 50 μM. The control group was given liposome without compounds on day 8. Mice were weighed everyday. Survival time was recorded in days after tumor administration. Antitumor activity was evaluated by comparing the mean survival time of the treated animals (T) with that of the controls (C) and by calculation of the ILS $(T/C - 1) \times 100$ (%).

Statistical Analysis and Presentation of Results. The statistical significance of the results from the survival experiment were assessed by nonparametric test of Kruskal-Wallis and Student's *t* test. Most experiments were repeated at least three times with consistent results.

RESULTS

Effects of Falconensone A and Its Derivatives on Growth of Various Cancer Cell Lines. We have studied the antitumor activities of falconensones (Fig. 1) as measured by their ability to suppress cell growth and induce differentiation and apoptosis in HL60 leukemia cells *in vitro* (2, 13). As a result, falconensone A and the falconensone A derivatives falconensone A *p*-bromophenylhydrazone and falconensone A dioxime were shown to be potent antitumor agents for HL60 cells, and falconensone B, lacking a methyl residue at the pentene ring 4′ position, showed a lack of activity. It was of question whether these compounds exhibited antitumor properties against HL60R, a subclone of HL60 cells, which is resistant to RA, as well as other cancer cell lines.

Initially, we examined time-dependent effects on HL60R cell growth by RA (1 μM) and falconensones (10 μM) to compare these results with results obtained against HL60 cell growth. RA was used as an internal standard. Growth of HL60 cells was inhibited at an earlier time point by falconensone A dioxime than by falconensone A or falconensone A *p*-bromophenylhydrazone (Fig. 2, *left panel*). At 21 h, cell growth was inhibited by ~21% for falconensone A and falconensone A *p*-bromophenylhydrazone, and by ~55% for falconensone A dioxime. At 43 h, falconensone A *p*-bromophenylhydrazone inhibited ~50% of cell growth as compared with ~23% for falconensone A and ~60% for falconensone A dioxime (Fig. 2, *left panel*). These results indicate that falconensone A and its derivatives inhibit HL60 cell growth in a time-dependent manner and that falconensone A dioxime was the most potent inhibitor of HL60 cell growth. In contrast, falconensone A and its derivatives suppressed growth of HL60R cells in a time-dependent manner, whereas RA had no effect (Fig. 2, *right panel*). At 24 h, falconensone A dioxime suppressed ~20% of cell growth. At 115 h, falconensone A and its derivatives inhibited ~65% of cell growth, whereas the shape of each growth inhibition curve was distinct (Fig. 2, *right panel*). These results suggest that falconensone A and its derivatives show antiprolif-
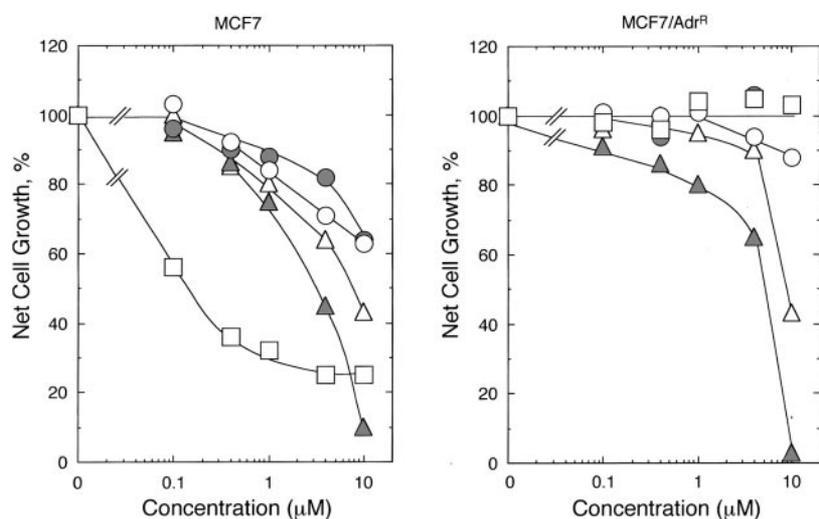
Fig. 3 Growth of MCF-7 and MCF-7/Adr[R] cells in the presence of falconensones. Cells were grown without or with various concentration of RA (□) and falconensones: falconensone A (○), falconensone B (◉), falconensone A *p*-bromophenylhydrazone (△), and falconensone A dioxime (▲) in medium containing 10% FBS. Growth was measured at 72 h as described under "Materials and Methods." Each *point* is the mean of at least four measurements. The SE of each point was ≤8% of the mean.

Fig. 4 Growth of HepG2 and DU-145 cells in the presence of falconensones. Cells were grown without or with various concentration of RA (□) and falconensones: falconensone A (○), falconensone B (◉), falconensone A *p*-bromophenylhydrazone (△), and falconensone A dioxime (▲) in medium containing 10% FBS. Growth was measured at 72 h as described under "Materials and Methods." Each *point* is the mean of at least four measurements. The SE of each point was ≤8% of the mean.
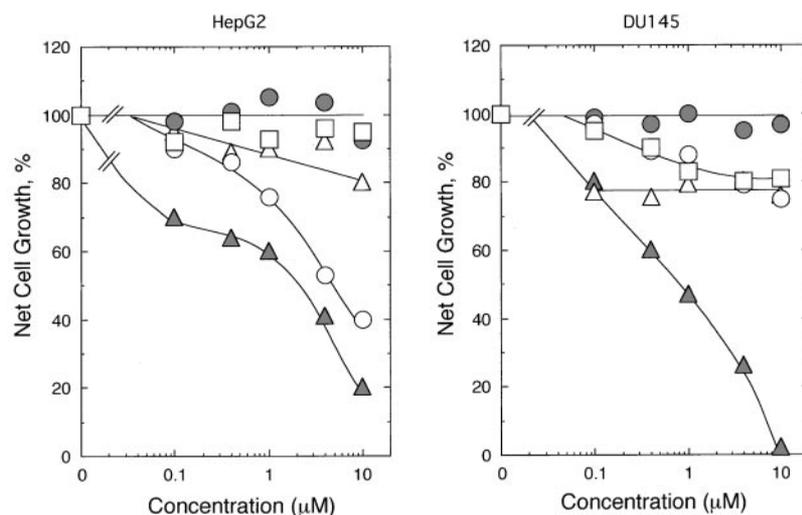

erative activity against both HL60 and HL60R cells, being more sensitive for HL60 cells rather than for HL60R cells. On the other hand, falconensone B did not measurably affect the growth of either HL60 or HL60R cells (Fig. 2).

MCF-7 cells, having estradiol receptors (ER-positive cells) or MCF-7/Adr[R] cells but not having estradiol receptors (ER-negative cells), were grown in the presence of various concentrations of RA and falconensones. All of the falconensones inhibited the growth of MCF-7 cells in dose-dependent manners (Fig. 3, *left panel*). At concentrations of 10 μM, cell growth was inhibited ~90% for falconensone A *p*-bromophenylhydrazone, ~60% for falconensone A dioxime, and ~37% for falconensone A and B. In contrast, against MCF-7/Adr[R] cells, which are resistant to RA, falconensone A *p*-bromophenylhydrazone and falconensone A dioxime (synthetic derivatives), showed much greater inhibitory effects on cell growth than did falconensone A and B (natural compounds; Fig. 3, *right panel*). At concentrations of 10 μM, falconensone A dioxime, falconensone A *p*-bromophenylhydrazone, and falconensone A, inhibited cell

growth by ~98%, 58%, and 11%, respectively, whereas falconensone B was inactive (Fig. 3, *right panel*). These results indicate that falconensone A dioxime and falconensone A *p*-bromophenylhydrazone are potent growth inhibitors against both human breast cancer cell lines, especially MCF-7/Adr[R] cells, which are resistant to RA.

Proliferation of HepG2 cells and DU-145 cells was inhibited in dose-dependent fashion by falconensone A dioxime, falconensone A *p*-bromophenylhydrazone, and falconensone A as shown in Fig. 4. At concentrations of 10 μM, falconensone A dioxime inhibited growth by ~80% against HepG2 cells and by ~98% against DU-145 cells (Fig. 4). In addition, falconensone A inhibited growth by ~60% against HepG2 cells and by ~20% against DU-145 cells. In contrast, falconensone B and falconensone A *p*-bromophenylhydrazone at concentrations of 10 μM did not measurably affect cell growth of either HepG2 or DU-145 (Fig. 4). These results suggest that falconensone A dioxime inhibits both HepG2 and DU-145 cell growth to a much greater extent than by other falconensones, and it was an effective
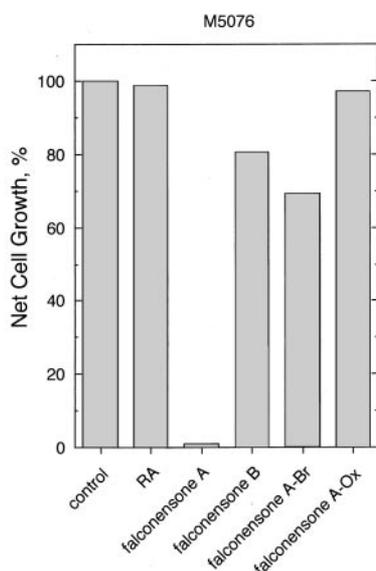
Fig. 5 Growth of M5076 cells in the presence of falconensones. Cells were grown without or with RA and falconensones at a concentration of 10 μM in medium containing 15% FBS. Growth was measured at 96 h as described under "Materials and Methods." The mean of at least three measurements are shown. The SE of each data point was ≤8% of the mean.

antiproliferative agent against liver and prostate cancer cell lines.

Murine reticulosarcoma M5076 cells were grown in culture containing 10 μM of falconensones and 15% FBS, which was required for exponential cell growth. As shown in Fig. 5, falconensone A inhibited ~98% of M5076 cell growth and was the most potent antiproliferative agent among the four falconensones. Cell growth was inhibited by ~31% for falconensone A *p*-bromophenylhydrazone and by ~19% for falconensone B, whereas falconensone A dioxime was inactive (Fig. 5). These results indicate that M5076 cell growth is suppressed by falconensone A more strongly as compared with other falconensones.

Survival Time of M5076-bearing Mice Treated with Falconensones. We found that falconensone A, falconensone A *p*-bromophenylhydrazone, and falconensone A dioxime exhibited anticancer activities to a much greater extent than falconensone B against various cancer cell lines *in vitro* and that the effectiveness of falconensones varied depending on cancer cell lines (Figs. 2–5). These results let us to investigate antitumor efficacy of these falconensones using a liver metastatic cancer model with M5076 cells. M5076 cells were injected via tail vein into mice on day 0, and then compounds encapsulated in liposomes and liposomes without compounds as control were administrated by i.v. on day 8. Final blood concentration of each compound was 50 μM.

Body weights of mice in all of the groups did not change significantly (data not shown). Shown in Fig. 6 are photographs of liver, heart, lung, kidney, and spleen of either normal mice or mice at 14 days after i.v. injection of M5076 cells. All of the tissues in the latter group were ~2-fold larger than those in

normal. White spots in the livers of M5076-bearing mice were seen and identified as liver tumors (16).

Fig. 7 shows effects of falconensones on survival time of M5076-implanted mice. All of the control mice had died by ~14 days after tumor implantation. In contrast, survival lines of mice treated with falconensones were prolonged and shifted to right in Fig. 7. The mean survival times of falconensone A, falconensone A *p*-bromophenylhydrazone, and falconensone A dioxime were 18.1, 21.3, and 23.3 days, respectively, as compared with control of 14.1 days (Table 1). The survival time differences between control and each falconensone were significant ($P < 0.001$). These results indicate that three falconensones showed significant antitumor activity. Percentage ILS values are 28.2% for falconensone A, 50.0% for falconensone A *p*-bromophenylhydrazone, and 64.1% for falconensone A dioxime (Table 1). Falconensone A dioxime exhibited the most potent antitumor efficacy, with one of nine mice surviving for over 28 days (Fig. 7).

DISCUSSION

The current study shows that the recently identified polyene, falconensone A isolated from *E. falconensis* and its derivatives, falconensone A *p*-bromophenylhydrazone and falconensone A dioxime, exhibit differential antiproliferative activities against human cancer cell lines, HL60, HL60R, HepG2, DU-145, MCF-7, and MCF-7/Adr^R cells *in vitro*. Falconensone B, the 4′-nor-methyl derivative of falconensone A, was inactive or exhibited low activity (Figs. 2–5). Cancer cell specificity among falconensones varied. In addition, survival times of M5076-implanted mice administrated with falconensone A or its derivatives were prolonged as compared with control mice (Fig. 7 and Table 1). In conclusion, falconensone A and its derivatives may suppress growth of a broad spectrum of cancer cells with high efficacy. These agents may have great potential for clinical use in the treatment of certain cancers.

Previous studies have shown that falconensone A, falconensone A *p*-bromophenylhydrazone, and falconensone A dioxime suppress growth and induce differentiation and apoptosis of HL60 cells, and are potent antioxidants *in vitro*, whereas falconensone B was found to be inactive (2, 4, 13). In the current study, falconensone B as well as the other falconensones suppressed proliferation of MCF-7 and M5076 cells (Figs. 3 and 5). Although we did not perform *in vivo* studies with falconensone B (Fig. 7 and Table 1), it would be of interest to investigate whether falconensone B exhibits anticancer efficacy *in vivo*.

Falconensone A showed greater growth suppression against M5076 cells than falconensone A *p*-bromophenylhydrazone and falconensone A dioxime, which were potent inhibitors of human cancer cell lines *in vitro* (Figs. 2–5). These three falconensones were also effective *in vivo* in the M5076-implanted mouse model (Fig. 7 and Table 1). It is possible that falconensone A may directly affect M5076 cell growth and that it and synthetic derivatives may affect metastatic cancer *in vivo*. Experiments are in progress to distinguish which step(s) are principally affected by each falconensone [*e.g.,* cell growth of M5076 (primary neoplasm), metastasis, or cell growth of metastatic cancer and so forth].
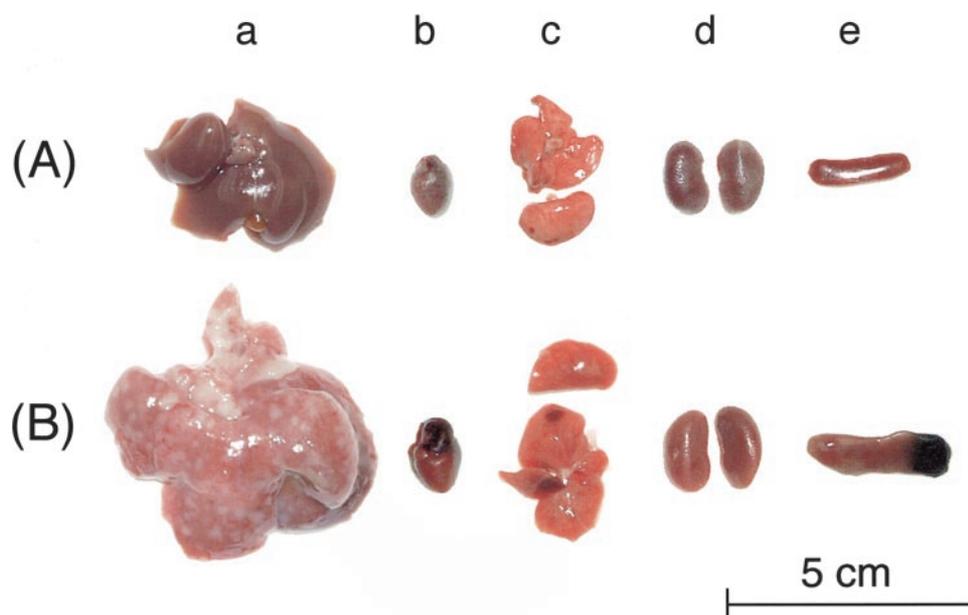
Fig. 6 Photographs of organs in normal or M5076-implanted mouse. Photographs of liver (*a*), heart (*b*), lung (*c*), kidney (*d*), and spleen (*e*) obtained from C57 BL/6J mouse (*A*) or mouse on day 15 after implanting with M5076 cells i.v. (*B*). Images were incorporated using Adobe Photoshop. *A,* normal mouse; *B,* mouse implanted with M5076 cells.
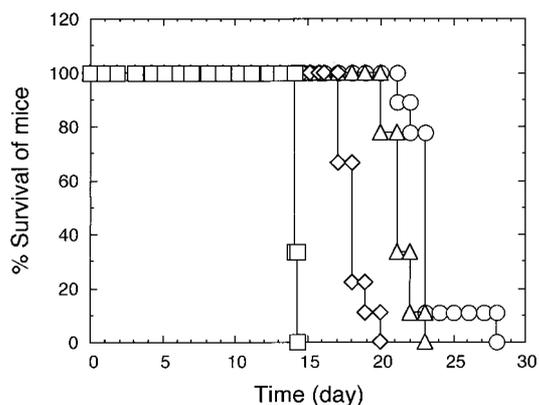

Fig. 7 Effects of liposomal falconensones on survival of M5076-implanted mice. Mice were weighed daily. Survival times was recorded in days after M5076 implanting (*n* = 9). Liposomal falconensone A (◇), falconensone A *p*-bromophenylhydrazone (△), falconensone A dioxime (○), and sterile saline (□).

Table 1 Antitumor efficacy of SG-liposomal falconensones against reticulum cell sarcoma (M5076)[a]

Preparation	Dose (μM)	Survival time (days)			% ILS[b]
		Mean	SD	Median	
Control		14.1	0.1	14.1	0.0
Falconensone A	50	18.1	1.0[c]	18.1	28.2
Falconensone A-Br	50	21.3	1.0[c]	21.1	50.0
Falconensone A-Ox	50	23.3	1.9[c]	23.1	64.1

[a] Antitumor activity was evaluated by comparing the mean survival time of the treated animals with that of the controls.

[b] Percentage ILS, $[(T/C - 1) \times 100 (\%)]$, where T and C represent the median survival time (days) of the treated and control animals, respectively.

[c] $P < 0.001$, significantly different from control.

The structures of falconensones, which have been determined by spectroscopic and X-ray analysis, have a novel carbon skeleton; namely, they are cyclopent-2-enone derivatives connected to an extensively conjugated methyl ketone located at the third methylene (C-3) as shown in Fig. 1. These compounds are hydrophobic and insoluble in water. Therefore, one of the reasons liposomes were used was to dissolve these hydrophobic drugs. Another reason was that liposomes are used to extend blood residence times so as to allow optimal delivery to target tissues. Liposomes stabilize components for long circulation times in blood (31). In addition, tumor targeting of drugs has been studied using liposomes consisting of ganglioside GM1 (32, 33) or amphipathic polyethylene glycol (34, 35) to avoid uptake by the reticuloendothelial system. Liposomes have also been modified with glycolipids and glycoproteins carrying galactose residues to target hepatocytes (36, 37). Recently it has been shown that liposomes (DPPC:SG:Ch = 6:1:3 molar ratio) having glucose residues were accumulated in liver by ~40% and in spleen by ~8% of total liposome at 2 h after i.v. injection (38). These were useful for effective liver targeting of drug delivery (29). Metastatic properties of M5076 murine reticulum cell sarcoma have also been evaluated. When these tumor cells were implanted by i.v., metastasis occurred preferentially to liver, ovary, spleen, and kidney regardless of the method of tumor injection (16, 17). In this latter study, falconensones encapsulated in liposomes containing SG prolonged survival of mice implanted with M5076 cells (Fig. 7 and Table 1). Falconensone A dioxime exhibited the most potent antitumor efficacy in this M5076 animal model, suggesting that falconensone A dioxime may affect metastasis or growth of metastatic cells, because it failed to inhibit M5076 cell growth directly (Fig. 5). Additional studies are needed to ascertain which process or steps each falconensone acts on.

Falconensone A *p*-bromophenylhydrazone shows antiproliferative activities against HL60, HL60R, HepG2, DU-145, MCF-7, MCF-7/Adr^R, and M5076 cells (Figs. 2–5), and affects survival of mice implanted with M5076 cells (Fig. 7). Of the current series, falconensone A *p*-bromophenylhydrazone is the most closely related to 4-HPR, a synthetic retinoid, which is an effective chemopreventive (6) and antiproliferative agent (7–9) against various types of breast, prostate, ovary, and bladder tumors. It is possible that falconensone A *p*-bromophenylhydrazone may be as potent as 4-HPR as an anticancer drug.

Clinically, patients with acute promyelocytic leukemia may initially respond to RA therapy with complete remission (39–42). However, relapses can be associated leukemia cells that are resistant to differentiation by RA (43). In the current study, falconensone A, falconensone A *p*-bromophenylhydrazone, and falconensone A dioxime inhibited growth of RA-resistant cell lines, HL60R and MCF-7/Adr^R (Figs. 2 and 3, *right panels*). Additional *in vivo* studies are required using falconensones as chemopreventive agents against acute promyelocytic leukemia.

Falconensone A and its derivatives are effective anticancer drugs in M5076 tumor models. The results reported herein may suggest potential clinical application of falconensone A and its derivatives in cancer patients. Studies are presently underway to measure the effectiveness of falconensone A and its derivatives against other animal tumor models and to evaluate mechanisms of biological activities of these agents.

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