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ORIGINAL ARTICLE

Antitumor activity of edotecarin in breast carcinoma models

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Abstract
Purpose Edotecarin (J-107088, formerly ED-749) is a potent indolocarbazole topoisomerase-I inhibitor that has the potential to treat solid tumors. The current studies evaluated the potency and antitumor activity of edotecarin, as a single agent and in combination with capecitabine or docetaxel.
Methods Antiproliferative activity was tested in vitro in a panel of 13 mammary cell lines and antitumor efficacy was tested in vivo in various breast cancer models.
Results Edotecarin inhibited cellular proliferation in breast carcinoma cell lines: 50% inhibitory concentrations ranged from 8 nmol/L in SKBR-3 cells to ~30 μmol/L in BT20 cells. Single dose and weekly intravenous treatments with edotecarin 30 and 150 mg/kg produced significant antitumor activity in the SKBR-3 human breast carcinoma xenograft model, with no major toxicities, compared with vehicle solvent treatment. Daily administration of edotecarin 15 mg/kg for 10 days was not well tolerated, whereas the total dose of 150 mg/kg was safe when administered in a single injection. Edotecarin 3 and 30 mg/kg given after docetaxel in the nude mouse SKBR-3 xenograft model produced tumor growth delays that were greater than those observed with either agent alone and with no toxicity as evaluated on the basis of body weight reduction (<20%). Furthermore, edotecarin 3 mg/kg in combination with capecitabine produced more than additive effects and the combination was well tolerated. However, edotecarin at a dose of 30 mg/kg in combination with capecitabine was lethal. Edotecarin also exhibited potent antitumor activity against xenografted human MX-1 cells, MMTV-v-Ha-ras oncogene-driven mouse breast tumors, and chemically induced rat mammary tumors.
Conclusions The data suggest that edotecarin may be useful as a single agent or a component of combination chemotherapy regimens for treating human breast cancer.

Keywords Edotecarin · Capecitabine · Docetaxel · Xenograft breast models · DMBA-induced mammary model · MMTV-v-Ha-ras transgenic model

Introduction

Breast cancer is one of the three most commonly diagnosed cancers among women in the United States and worldwide [1]. In 2006, breast cancer is expected to account for 32% of all new cancer cases in women. Although the mortality rate from breast cancer in women continues to decrease, there remains a great need for more effective therapeutic options, especially for advanced stages of this disease.

Topoisomerase enzymes are essential for all DNA transactions; several topoisomerase inhibitors have a wide range of antitumor activities. Topoisomerase-I inhibitors are among the most widely used anticancer drugs clinically [2]. Camptothecin, an alkaloid that was originally isolated from the Chinese tree *Camptotheca*

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acuminata, was the first specific topoisomerase-I inhibitor to have clinical application [2–4]. Its primary mechanism of action is binding to the topoisomerase-I–DNA complex [5], thereby blocking DNA replication and ultimately leading to apoptosis and cell death [4].

Water-soluble analogues of camptothecin that have superior antitumor activities and acceptable toxicity profiles have been developed [4, 6]. Among these analogues, irinotecan [2, 4], topotecan [5], and 9-aminocamptothecin (9-AC) [2, 7] have shown significant clinical activity.

The indolocarbazole edotecarin is a potent noncamptothecin topoisomerase-I inhibitor with the potential for use in treating a number of solid tumors [8]. The chemical structure of edotecarin differs from other topoisomerase-I inhibitors that are in clinical use or undergoing clinical investigation [9]. The DNA complexes formed by edotecarin have been shown to be more stable than those formed by camptothecin. Furthermore, edotecarin has been shown to be a more potent inducer of single-strand DNA cleavage by topoisomerase I than camptothecin, with an effective concentration value of 0.05 μmol/L, compared with 0.42 μmol/L for camptothecin [8]. Thus, edotecarin may not have some of the limitations of existing topoisomerase-I inhibitors. The drug has been found to exhibit significant antitumor activity in vivo in human tumor models of LX-1 lung, PC-3 prostate, and central nervous system tumor-derived nude-mouse xenografts [10]. Furthermore, clinical trials in humans have established that edotecarin has activity as a single agent in patients with metastatic colorectal cancer who have been heavily pretreated [8].

The current studies explored the activity of edotecarin in rodent model systems for breast cancer development: the MMTV-v-Ha-ras oncogene-driven mouse mammaty model and the carcinogen-induced rat breast tumor model. In addition, edotecarin was also tested as a single agent in SKBR-3 and MX-1 human breast cancer xenograft models.

Because antitumor drugs at their maximally suitable doses do not have significant selective cytotoxicity and combination therapy is commonly used in the treatment of cancer [11], antitumor effect of edotecarin was also evaluated in combination with other agents. Specifically, these studies examined the antitumor efficacy of edotecarin in combination with docetaxel, the most effective single-agent second-line treatment for metastatic breast cancer, and in combination with capecitabine, an oral fluoropyrimidine carbamate, which is converted to 5-fluorouracil selectively in tumors [12]. Capecitabine is approved as treatment for anthracycline- and taxane-pretreated metastatic breast cancer patients [13]. Edotecarin, when administered either as a single agent or in combination chemotherapy, was found to produce marked antitumor efficacy in these model systems of human breast cancer.

Materials and methods

Cell lines

An in vitro panel of 13 mammary cell lines was obtained from American Type Culture Collection (Manassas, VA, USA). Human breast carcinoma xenografts SKBR-3 (from an in vitro cell line) and MX-1 (obtained from the National Cancer Institute, Frederick, MD, USA) were maintained by subcutaneous transplantation in athymic mice using tumor brei 20–30 mg. For the in vivo protocols, tumors were excised, and fragments were implanted subcutaneously in the left flank. Treatments were started when the tumors became measurable. Mean tumor weight in all groups was 0.175–0.180 g.

Animals

Balb-nu/nu female mice, obtained from Harlan Italy (San Pietro al Natisone, Italy), were housed under pathogen-free conditions in micro-isolator cages, with irradiated rodent chow and water available ad libitum. MMTV-v-Ha-ras transgenic mice, as previously described [14], were obtained from Charles River Breeding Laboratories, Inc. (Calco, Italy). Female Sprague–Dawley (IOPS OFA strain) rats aged 7 weeks were obtained from Iffa-Credo (Saint-Germain sur L'Arbresle, France). The rats were housed two per cage, with rodent chow and water available ad libitum.

All animal studies were performed using procedures in compliance with Italian Legislative Decree N. 116, 27 January 1992, enforcing European Communities Council Directive N. 86/609/EEC concerning the protection of animals used for experimental or other scientific purposes, and according to institutional policy regarding the care and use of laboratory animals.

Test compounds

All compounds were prepared immediately before use. Edotecarin was dissolved with 20% of polyethylene glycol (PEG) 400 in water. Irinotecan was dissolved in glucosate water. The pharmacy preparation of docetaxel was diluted in glucosate water; the pharmacy preparation of capecitabine was disrupted and resus-

pended in Methocel® (The Dow Chemical Company, Midland, MI, USA) and administered orally.

Tumor growth evaluation

Tumor growth was assessed with Vernier calipers, and the two diameters were recorded. Tumor volume was calculated according to the following formula: length (mm) × width^2 (mm)/2. Tumor growth inhibition was calculated as follows: 100 - (mean tumor weight of treated group/mean tumor weight of control group) × 100.

Evaluation of antitumor activity in xenograft models

Tumor growth and net body weight of the mice were evaluated every 3 days. The effectiveness of the anticancer treatment was determined as the delay in the onset of exponential growth of the tumor according to the criteria of Bissery et al. [15]. Tumor growth delay was defined as the difference between the treatment and the control group in the median time (in days) for the tumor to reach the predetermined size of 1 g. Toxicity was evaluated on the basis of loss of body weight. Mice were sacrificed when the tumor achieved a volume that hampered activity. Gross autopsy findings—mainly reductions in spleen and liver size—were reported. Mice that were tumor free 90 days after implantation were considered cured.

Evaluation of antitumor activity in MMTV-v-Ha-ras transgenic mice

Administration of a compound began when mammary tumors in MMTV-v-Ha-ras mice were determined to have reached an average volume of 300–500 mm^3. Test groups consisted of 6 or more mice. Tumor growth was measured approximately twice a week.

At the end of the 4-week treatment period, tumor response to the drug was assessed and classified as complete remission (disappearance of the tumor), partial remission (>50% reduction of initial tumor weight), stable disease (<50% increase or decrease in tumor weight), or progression (>50% increase in tumor weight).

Evaluation of antitumor activity in dimethylbenzanthracine (DMBA)-induced mammary tumors

Dimethylbenzanthracine and its vehicle—sesame oil—were purchased from Sigma–Aldrich (St. Louis, MO, USA). Female 50-day-old Sprague–Dawley rats were intubated with a single intragastric dose of DMBA 20 mg in sesame oil 1 mL, as previously described [16]. After 40 days, rats were examined weekly by palpation. When more than one mammary tumor measuring 1 cm in diameter was identified, rats were divided sequentially into test groups of ≥10 rats per group. Tumor volume was measured weekly. Tumor response to the drug was evaluated as described for the MMTV-v-Ha-ras transgenic mouse model.

Drug administration studies

Edotecarin was either administered as a single dose or given once weekly for 4 weeks at doses of 30 and 150 mg/kg, previously reported as active and non toxic doses [17]. Edotecarin was also tested at 15 mg/kg daily for 5 consecutive days repeated for 2 weeks. In combination studies edotecarin was administered in two doses (3 and 30 mg/kg). The second drug, docetaxel or capecitabine, was administered at 70% of the maximum tolerated dose (MTD) and at the optimal schedule. MTD and optimal schedules were identified on data from separate unpublished internal studies. Docetaxel was administered three times at doses of 5 mg/kg every 4 days starting 24 h before the first injection of edotecarin. Capecitabine was administered at a dose of 370 mg/kg starting 24 h after the first injection of edotecarin and continuing daily for 14 days. The treatment schedule for MX-1 breast xenograft tumor models was six doses of edotecarin 150 mg/kg every 4 days, irinotecan 60 mg/kg, or vehicle. MMTV-v-Ha-ras mice were treated weekly with either edotecarin 100 mg/kg intravenously or its vehicle for 4 consecutive weeks, followed by cessation of dosing to monitor tumor regrowth. For DMBA, rats were examined weekly by palpation after 40 days. When more than one mammary tumor measuring 1 cm in diameter was identified, rats were placed sequentially into two groups and treated weekly for 4 consecutive weeks with edotecarin 10 mg/kg intravenously or vehicle alone. The 10-mg/kg dose of edotecarin in these rats is approximately equivalent to the efficacious 20-mg/kg dose in mice based on allometric scaling.

Statistical analyses

The Mann–Whitney *U*-test was used to determine differences between treatment groups. Analysis using GraphPad Prism® (GraphPad Software Inc., San Diego, CA, USA) was performed of all evaluations of tumor weight and for the day of maximum net body weight loss. A $P<0.05$ was considered statistically significant.

Results

In vitro inhibition of mammary cell lines by edotecarin and SN-38

Determination of 50% inhibitory concentrations was performed using various breast cancer cell lines. The 50% inhibitory concentrations of edotecarin ranged from 8 nmol/L in SKBR-3 cells to ~30 μmol/L in BT20 cells. The potency of edotecarin generally paralleled that of SN-38, the active metabolite of irinotecan (Table 1).

SKBR-3 xenograft model

Because edotecarin was found to have potent cytostatic activity against the human breast carcinoma cell line SKBR-3 in vitro, further experiments were performed with this cell line in xenografted tumors of nude mice. Edotecarin as a single agent was found to be active against the human SKBR-3 breast carcinoma xenograft tumor model (Table 2). Single treatments of edotecarin 30 and 150 mg/kg produced significant mean tumor growth delays of 9.8 and 14.9 days, respectively. A proportional improvement in antitumor activity was observed with a regimen of four once-a-week treatments of edotecarin; doses of 30 and 150 mg/kg delayed tumor growth 29 and 46.5 days, respectively. No major toxicity was observed, and net body weight reduction was <20%. The results obtained with 30 mg/kg edotecarin weekly for 4 weeks were comparable to that obtained with 45 mg/kg irinotecan at its most efficacious schedule of administration (every 4 days for six treatments).

Table 3 lists the results with edotecarin in combination with docetaxel or capecitabine in the SKBR-3 human breast carcinoma model compared with the compounds as single agents. Combination chemotherapy with edotecarin and docetaxel produced an effect on tumor growth delay that was better than additive, because the delay was longer than the sum of the delay times for the compounds when given as single agents at the same doses (20.3 and 30.6 days for the combination versus 10.5 and 22.4 days when delays with docetaxel were added to those with edotecarin 3 and 30 mg/kg, respectively). Statistical analysis of the data performed on tumor weight for days 29 and 38 confirmed a significant improvement in the combination groups versus the groups treated with single doses of docetaxel and edotecarin at the same dosage ($P < 0.005$).

The combination of capecitabine and edotecarin 3 mg/kg resulted in a tumor growth delay of 34.9 days compared with the expected additive delay of 29.5 days. Statistical analysis of the data on tumor weight for days 29 and 38 confirmed the greater improvement in the group treated with the combination than in the group treated with capecitabine or edotecarin as a single agent ($P < 0.01$). It should be noted, however, that the combination of capecitabine with edotecarin 30 mg/kg resulted toxic in all mice: five died (body weight reduction >20%) and three were sacrificed.

MX-1 model

A comparison of edotecarin and irinotecan at the maximal tolerated doses (150 and 60 mg/kg, respectively)

Table 1 In vitro cytotoxic activity in mammary cell lines

Cell line	IC_{50} (μmol/L)[a]	
	Edotecarin	SN-38
BT20	29.25 ± 1.97	0.17 ± 0.04
BT474	13.85	0.82 ± 0.18
BT549	0.03 ± 0.01	0.02 ± 0.02
HS578	0.66	0.85
MCF7	0.85 ± 0.24	0.06 ± 0.04
MDA-MB-134	0.53 ± 0.30	0.19 ± 0.02
MDA-MB-231	0.12 ± 0.12	0.13 ± 0.09
MDA-MB-361	0.05 ± 0.04	0.03 ± 0.00
MDA-MB-435	0.47 ± 0.22	0.10 ± 0.03
MDA-MB-453	0.06 ± 0.00	0.01 ± 0.00
MDA-MB-468	0.01 ± 0.01	0.006 ± 0.004
SKBR-3	0.008 ± 0.003	0.004 ± 0.003
T47D	0.04 ± 0.00	0.009 ± 0.007

[a] IC_{50}: 50% inhibitory concentration was calculated after 72-h treatment. Cell proliferation was evaluated by the CellTiter-Glo® Luminescent Cell Viability Assay (Promega Italia srl., Milano, Italy)

Table 2 Antitumor efficacy of different schedules of edotecarin in the SKBR-3 human breast carcinoma model

Treatment	Tumor weight, g ± SD (day 24)	TI, % (day 24)	Time to reach 1 g (day)	T-C (day)	Toxicity (*n*)
Control	3.34 ± 0.27	0	15.1		0/8
Irinotecan 45 mg/kg q4d x6	0.54 ± 0.06	84	39.5	24.4	0/8
Edotecarin 30 mg/kg single dose	0.76 ± 0.09	77	24.9	9.8	0/8
Edotecarin 30 mg/kg q7d x4	0.26 ± 0.04	92	44.1	29	0/8
Edotecarin 150 mg/kg single dose	0.37 ± 0.07	89	30.0	14.9	0/8
Edotecarin 150 mg/kg q7d x4	0.17 ± 0.03	95	61.6	46.5	0/8

SD standard deviation, *TI* tumor growth inhibition, *T-C* tumor growth delay

Table 3 Antitumor efficacy of edotecarin in combination with docetaxel or capecitabine in the SKBR-3 human breast carcinoma model

Treatment	Tumor weight, g ± SD (day 24)	TI, % (day 24)	Time to reach 1 g (day)	T-C (day)	Toxicity (*n*)
Control	3.05 ± 0.24	–	13.2	–	0/8
Edotecarin 3 mg/kg[a]	1.30 ± 0.12	57	19.6	6.4	0/8
Edotecarin 30 mg/kg[a]	0.54 ± 0.07	82	31.5	18.3	0/8
Docetaxel 5 mg/kg[b]	1.60 ± 0.29	48	17.3	4.1	0/8
Docetaxel 5 mg/kg[b]/edotecarin 3 mg/kg[a]	0.48 ± 0.05	84	33.5	20.3	0/8
Docetaxel 5 mg/kg[b]/edotecarin 30 mg/kg[a]	0.35 ± 0.05	89	43.8	30.6	0/8
Capecitabine 370 mg/kg[c]	0.82 ± 0.08	73	36.3	23.1	0/8
Edotecarin 3 mg/kg[a]/capecitabine 370 mg/kg[c]	0.49 ± 0.04	84	48.1	34.9	0/8
Edotecarin 30 mg/kg[a]/capecitabine 370 mg/kg[c]	–	–	–	–	8/8[d]

SD standard deviation, *TI* tumor growth inhibition, *T-C* tumor growth delay

[a] Intravenous treatment on days 9 and 16

[b] Intravenous treatment on days 8, 12, and 15

[c] Oral treatments from days 10 to 23

[d] The 5/8 mice died, and their organs were found to be reduced at gross autopsy 3/8 were sacrificed on day 20 due to suffering

was carried out in the MX-1 breast xenograft tumor model [18]. In the current study, after 95 days, eight of eight mice were tumor free with both treatments.

MMTV-v-Ha-ras transgenic mouse model

In the MMTV-v-Ha-ras transgenic mouse model, seven mice treated with vehicle demonstrated rapid tumor progression (Fig. 1), differently from those treated with edotecarin. At the end of the 4-week treatment period, four mice treated with edotecarin had partial remission, two had complete remission, and one had stable disease.

DMBA-induced rat breast tumor model

Tumor growth in rats treated with edotecarin was reduced in the DMBA-induced rat breast tumor model (Fig. 2). Of rats treated with vehicle, 14 had disease progression, whereas of those treated with edotecarin, 10 had progression, 1 had partial remission, and 3 had stable disease. Body weight reduction <10% was observed in treated rats, this indicating that also a higher dose of edotecarin could be safely administered in order to observe an higher antitumor efficacy.

Discussion

In the current studies, the antitumor effects of edotecarin as a single agent and in combination with docetaxel or capecitabine were examined. Edotecarin when used as a single agent, although slightly less active than SN-38, showed potent cytostatic activity in vitro in a panel of 13 breast cancer cell lines: seven cell lines were highly sensitivity (IC_{50} ≤0.1 μmol/L) to edoteca-

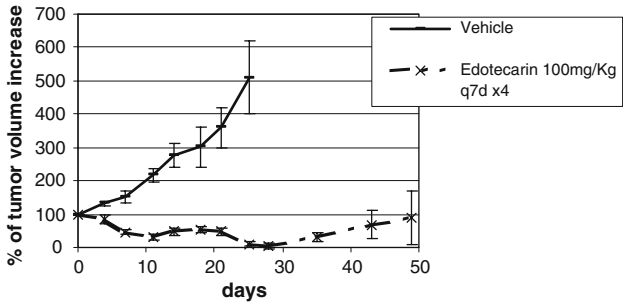

Fig. 1 Tumor volume in MMTV-v-Ha-ras transgenic model in mice treated with vehicle or edotecarin. Administration of a compound began when mammary tumors in MMTV-v-Ha-ras mice reached an average volume of 300–500 mm^3. Mice were treated weekly for 4 consecutive weeks with edotecarin 100 mg/kg intravenously or vehicle alone. Tumor growth was measured approximately twice a week and the percentage of tumor volume increase from the beginning of the treatment was calculated

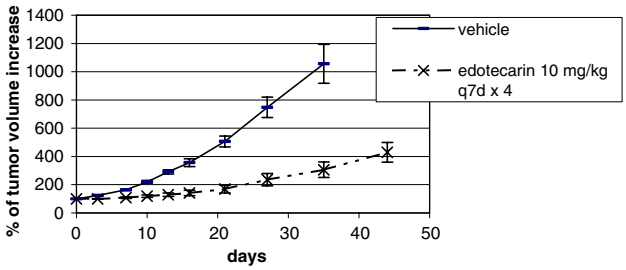

Fig. 2 Tumor volume in DMBA-induced mammary tumor in rats treated with vehicle or edotecarin. Rats were examined weekly by palpation after 40 days from DMBA treatment. When more than one mammary tumor measuring 1 cm in diameter was identified, rats were placed sequentially into two groups and treated weekly for 4 consecutive weeks with edotecarin 10 mg/kg intravenously or vehicle alone. Tumor volume was measured weekly and the percentage of tumor volume increase from the beginning of the treatment was calculated

rin whereas nine cell lines were highly sensitive to SN-38; four cell lines showed an intermediate sensitivity (IC_{50}: 0.1–1 μmol/L) to both compounds; two cell lines (BT20 and BT474) were resistant to edotecarin (IC_{50} >10 μmol/L). The lesser sensitivity of some cell lines to edotecarin may have been due to overexpression of the ATP-binding cassette transporter BCRP/MXR/ABCP, which has been demonstrated to affect the drug's activity [19]. For example, it has been reported that the BCRP mRNA expression level in BT20 cells is 22-fold higher than in T47D cells and sixfold higher than in SKBR-3 cells [20].

Comparisons of edotecarin and irinotecan were performed on two estrogen receptor—negative breast xenograft models, SKBR-3 [21] and MX-1 [22]. In both models, the antitumor activity of both drugs as single agents was shown to be comparable. Weekly treatments of edotecarin 150 mg/kg showed significant antitumor activity against the SKBR-3 human breast carcinoma model, with no major toxicity, and cured all the animals in the MX-1 model, that was previously reported to be sensitive to this drug, in fact treatments twice a week for 2 consecutive weeks produced a 75% tumor growth inhibition [17].

In combination studies, edotecarin 3 or 30 mg/kg exhibited more than additive activity when administered with docetaxel, and the combination was well tolerated. In combination with capecitabine, edotecarin 3 mg/kg also had a more than additive effect. However, edotecarin 30 mg/kg combined with capecitabine was lethal. On these findings we can speculate that the combination of compounds acting on the same cellular process, such as edotecarin and capecitabine, both active on DNA metabolism and synthesis, could have a synergistic toxic effect on normal proliferating cells higher than the combination of drugs with a different mode of action.

The antitumor efficacy of edotecarin was also observed in an oncogene-driven mammary model, MMTV-v-Ha-ras, which demonstrated the efficacy of edotecarin against hormonally driven expression of an oncogene in mammary tissues. Reduction in tumor growth was also observed in a carcinogen-induced, hormone-responsive rat mammary tumor model [23, 24].

In summary, these studies demonstrate that edotecarin exhibits potent antitumor activity in human xenograft breast cancer models and in chemically and hormonally influenced oncogene-induced mammary tumors. Furthermore, this agent enhances the effects of docetaxel and capecitabine in human breast cancer xenografts. Thus, edotecarin, either as monotherapy or in combination with established treatment modalities, is a potentially effective therapeutic option for patients with breast cancer, independent of their estrogen receptor status.

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