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SHORT COMMUNICATION

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Growth-inhibitory effects of the synthetic retinoid CD437 against ovarian carcinoma models in vitro and in vivo

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Abstract The activity of CD437{6-[3-(1-adamantyl)-4 hydroxyphenyl]-2-naphthalene carboxylic acid}, a relatively selective activator of RAR-γ, was evaluated against four human ovarian-carcinoma cell lines : PE01, PE04 (a Pt-resistant in vivo-derived counterpart of PE01), $PE01^{CDDP}$ (a Pt-resistant in vitro-derived model of PE01) and PE014. Growth inhibition was observed after 3 and 6 days of exposure to sub-micromolar concentrations as assessed by a reduction in cell number. IC_{50} values against PE01, PE04, $PE01^{CDDP}$ and PE014 were 0.09, 0.21, 0.12 and 0.28 μ*M* (day 3) and 0.1, 0.14, 0.07 and 0.17 μ*M* (day 6), respectively. Cisplatin-resistant cell lines were as responsive as cisplatin-sensitive lines, indicating potential activity in resistant disease. CD437 was also evaluated against the PE04 xenograft grown in nude mice using daily doses of 20 (days 0–4) and 10 mg/kg (days 0–4 and 7–11) given either by i.p. delivery or oral administration. Significant growth inhibition ($P < 0.05$) was obtained for both doses and by both routes. These data provide further support for the view that retinoids have value for the treatment of ovarian cancer.

Key words CD437 · Retinoid · Ovarian cancer Xenograft

Introduction

There is increasing interest in the use of retinoids in cancer therapy [1], and previous reports suggest that retinoids demonstrate antiproliferative activity against ovarian-cancer cell lines in vitro [2–8]. These studies also demonstrated that growth inhibition was associated with both apoptosis and increased differentiation. To date, only a single report has been published on the activity of a retinoid against ovarian cancer in vivo and this describes the positive activity of fenretinide against a human ovarian cancer xenograft alone and in combination with cisplatin, the major first-line drug used in the treatment of ovarian cancer [9]. Retinoid signal transduction is mediated via a network of six interacting nuclear receptors that fall into two classes, the RARs (α, β and γ) and the RXRs (α, β and γ) [10, 11]. The novel retinoid CD437{6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid} is a relatively selective activator of RAR-γ [12] and has shown antiproliferative activity against breast carcinoma, leukemia and melanoma systems [13–17]. In a recent study using non-small-cell lung-cancer cell lines (non-SCLC), CD437 was the most potent of 37 natural and synthetic retinoids and was the only retinoid active at submicromolar concentrations [18]. The present study was initiated to evaluate the activity of CD437 against (1) a panel of human ovarian-carcinoma cell lines in vitro, including both cisplatin-sensitive and cisplatin-resistant models, and (2) an ovarian carcinoma xenograft.

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Materials and methods

Drugs

CD437 was supplied by CIRD Galderma in lyophilised form and was kept at 4 °C until use. It was dissolved in dimethylsulfoxide (DMSO) for preparation of a stock solution (30 m*M*), and aliquots were kept at −40 °C.

Cell lines

The ovarian-cancer cell lines PE01, PE04, PE014 and $PE01^{CDDP}$ were used in this study [19]. Brief characterisation details are given in Table 1. Cell lines were routinely maintained in 5% fetal calf serum (FCS)/95% RPMI 1640.

Table 1 Characteristics of the cell lines and xenograft evaluated in the present study (*5-FU* 5-fluorouracil)

	Pathology of primary tumour	Prior treatment (of patient)	Comments
Cell line:			
PE01	Poorly differentiated serous carcinoma	Cisplatin/5-FU/chlorambucil	Sensitive to cisplatin
PE04	Poorly differentiated serous carcinoma	Cisplatin/5-FU/chlorambucil	Developed cisplatin resistance in patient
$PE01^{CDDP}$	Poorly differentiated serous carcinoma	Cisplatin/5-FU/chlorambucil	Made cisplatin-resistant in vitro
PE014	Well-differentiated serous carcinoma	No treatment	
Xenograft:			
PE04	Poorly differentiated serous carcinoma	Cisplatin/5-FU/chlorambucil	In vivo counterpart of cell line

In vitro experiments

Cells were grown to the late log phase in 175 -cm^2 flasks containing RPMI 1640 supplemented with 5% heat-inactivated FCS, 2 m*M* glutamine and antibiotics (penicillin/streptomycin). The cells were trypsinised, syringed to produce a single-cell suspension and plated in 24-well trays at a density of 2.5×10^4 cells/well for the PE01 and $PE01^{CDDP}$ cell lines and 5×10^4 cells/well for the PE04 and PE014 lines.

After 48 h the medium was removed and replaced with fresh medium containing CD437. Each dilution of CD437 and the untreated control contained a final concentration of 0.1% DMSO. Cells were counted on days 3 and 6 on a Coulter counter after trypsinisation; day-6 cells were refed with medium containing CD437 after 72 h of exposure. Graphs of cell growth and cell kill were plotted, from which IC_{50} values were determined.

In vivo experiments

Female nude mice were obtained from Harlan OLAC (Oxford) and were housed in negative-pressure isolators. Mice were at least 8 weeks old at the time of experimentation. The PE04 xenograft had been initiated by s.c. implantation of 10^7 cells of the cultured cell line into the flanks of nude mice [20]. Tumour fragments were implanted s.c. into both flanks of mice. After 4–6 weeks, when tumours had reached a mean volume of 50 mm^3, animals were allocated to treatment and control groups (each containing five mice) and treatment was commenced (defined as day 0). CD473 was emulsified in 25% cremophor EL/75% water and was given orally or by i.p. injection. The 20 -mg/kg daily dose was given on days 0–4, whereas 10 -mg/kg daily dose was given on days 0–4 and 7–11. Tumour dimensions were measured by calipers and volumes were calculated by the formula $v = \pi/6 \times l \times w^2$. Relative tumour volumes were then assessed by division of the volume determined on a particular day by the volume calculated at the initiation of treatment (day 0).

Results

The effects of CD437 on the growth in culture of four ovarian-carcinoma cell lines (PE01, $PE01^{CDDP}$, PE04 and PE014) were first determined. The compound was tested at concentrations ranging between 0.01 and 1 μ*M*, and mean IC_{50} values obtained from three independent groups of experiments are recorded in Table 2. CD437 demonstrated concentration-dependent growth inhibition, with IC_{50} values against PE01, PE04, $PE01^{CDDP}$ and PE014 being 0.09, 0.21, 0.12 and 0.28 μ*M* on day 3 and 0.1, 0.14, 0.07 and 0.17 μ*M* on day 6, respectively. These results indicate that CD437 is growth-inhibitory at submicromolar concentrations in this series of ovarian-carcinoma cell lines. The cisplatin-resistant PE04 and $PE01^{CDDP}$ cell lines were as sensitive to CD437 as were the PE01 and PE014 cell lines.

Table 2 Growth-inhibitory concentrations of CD437 against ovarian-cancer cell lines[a]

Cell line	Mean IC_{50} value (*M*)	
	Day 3	Day 6
PE01	$0.9 \pm 0.1 \times 10^{-7}$	$1.0 \pm 0.4 \times 10^{-7}$
PE04	$2.1 \pm 0.2 \times 10^{-7}$	$1.4 \pm 0.1 \times 10^{-7}$
$PE01^{CDDP}$	$1.2 \pm 0.4 \times 10^{-7}$	$0.7 \pm 0.1 \times 10^{-7}$
PE014	$2.8 \pm 0.3 \times 10^{-7}$	$1.7 \pm 0.6 \times 10^{-7}$

[a] Data represent mean values ± SD recorded for 3 independent experiments. All 4 cell lines were inhibited by submicromolar concentrations of CD437

CD437 was then tested against the PE04 ovarian carcinoma xenograft at a daily dose of 30 mg/kg as based on a previous study of CD437 against a melanoma xenograft that had investigated doses of 10 and 30 mg/kg given daily [16]. Significant growth inhibition was obtained with CD437 (100% inhibition on day 3 and 71% inhibition on day 7) at this dose, but one of five animals died (data not shown). A second experiment then assessed the effects of 20 mg/kg given daily on days 0–4 and of 10 mg/kg given daily on days 0–4 and 7–11 on tumours with a mean volume of 50 mm^3 (Fig. 1). The i.p. and oral routes of CD437 administration were compared and both doses given via either route demonstrated significant growth inhibition by day 3 with no lethality or body weight loss. The 5-day schedule using a daily dose of 20 mg/kg given orally produced 74% and 66% inhibition on days 7 and 14, respectively, whereas i.p. dosing produced 53% and 48% inhibition on the same days. The 10-day schedule using a daily dose of 10 mg/kg produced 71% and 66% inhibition on days 7 and 14, respectively, on oral dosing and 80% and 68% inhibition, respectively, following i.p. injection.

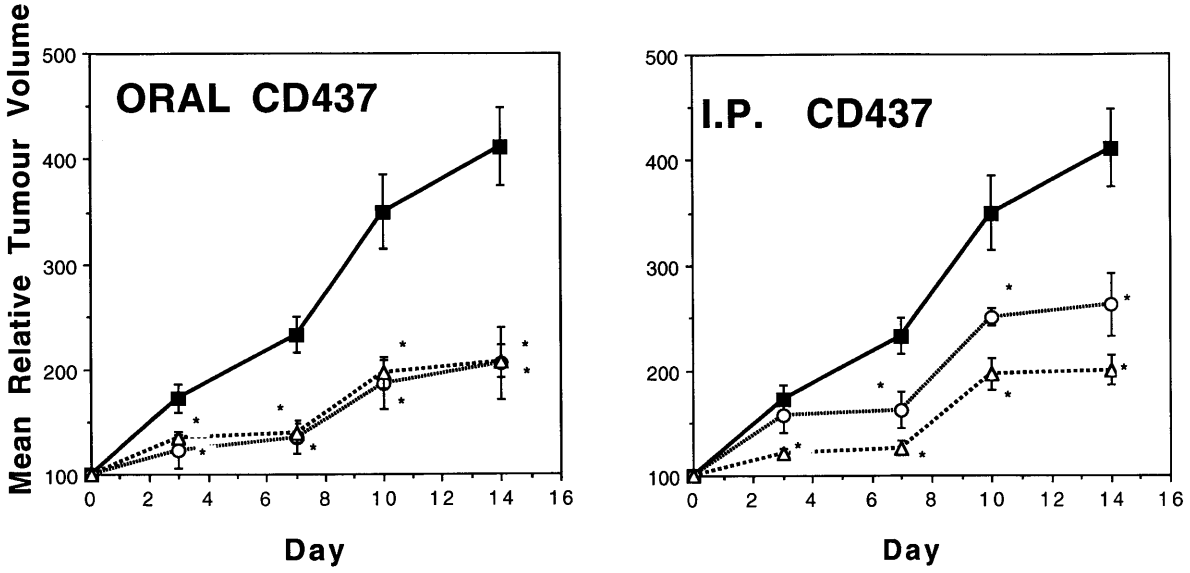

Fig. 1 Growth inhibition observed in the PE04 ovarian carcinoma xenograft following treatment with CD437. Mice with advanced (approximately 50 -mm^3 s.c. PE04 xenografts were given CD437 by oral or i.p. administration. Control (■–■), 20 mg/kg (○–○) given daily on days 0–4, 10 mg/kg (△–△) given daily on days 0–4 and 7–11). Data represent mean values ± SEM (n = 7 or 8 tumours). *$P < 0.05$ versus the control (Student's t-test)

Discussion

The in vitro data obtained in this study indicate that CD437 is active at submicromolar concentrations against a range of ovarian-carcinoma cell lines, with IC_{50} concentrations being approximately 10^{-7} M. These values are similar to those obtained with CD437 against melanoma and non-SCLC cell lines, which are inhibited at IC_{50} values ranging from 10^{-7} to 10^{-6} M, depending on the cell line evaluated [16–18]. Comparable effects of *cis*-retinoic acid in the PE04 cell line are obtained at concentrations of > 30 μM [2], indicating that CD437 is > 100 times more potent as a growth inhibitor against this cell line. A previous comparison of CD437 against 36 other retinoids indicated the marked potency of this compound in contrast to other retinoids, and the present results are consistent with this observation [18]. Since CD437 was active against both cisplatin-sensitive and cisplatin-resistant ovarian cancer models, it could also have value against disease that has become resistant after clinical treatment.

CD437 demonstrated significant activity against the PE04 xenograft, with similar activity being obtained by the oral and i.p. routes. CD437 has previously shown a comparable level of activity against the MeWo melanoma xenograft; in that study a daily dose of 30 mg/kg had produced activity similar to that obtained with a daily dose of 10 mg/kg and the magnitude of the drug's oral activity was indistinguishable from that of its intratumoural activity [16]. These previously published data, together with our own, suggest that relatively small changes in dose do not produce large differences in antitumour efficacy and that the route of administration is not critical. This contrasts with data obtained in the only other report published on the study of a retinoid tested against a human ovarian xenograft, in which fenretinide [N-(4-hydroxyphenyl) retinamide] was active when given by the i.p. route but not by the oral route [9].

We did not investigate the mechanism of action of CD437 in this study, but previous reports have indicated that the retinoid induces apoptosis both in vitro and in vivo [13–16]. The drug increases expression of $p21^{WAF1/CIP1}$ and GADD45 in breast carcinoma and leukemia cells in a p53-independent manner [13, 14] and down-regulates the expression of a number of proteins, including bcl-X, that antagonize apoptosis [15]. Recent data also suggest that CD437 can induce apoptosis in cells that are resistant to *all-trans*-retinoic acid and that this effect may be mediated by a mechanism that is independent of transactivation of retinoid receptors or transrepression of activator protein-1 (AP-1) [21]. In conclusion, these data indicate that CD437 has efficacy in ovarian carcinoma systems and provides further support for the view that retinoids may have value for the treatment of ovarian cancer.

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