Antitumor activity of the combination of synthetic retinoid ST1926 and cisplatin in ovarian carcinoma models

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Background: The novel adamantyl retinoid ST1926 is a potent inducer of apoptosis in ovarian carcinoma cells. Since the pro-apoptotic effect is associated with activation of p53, in this study we have investigated the efficacy of combination of ST1926 with cisplatin, a DNA-damaging agent that is known to induce p53-dependent apoptosis.

Materials and methods: The efficacy of ST1926 and its combination with cisplatin was evaluated in human ovarian carcinoma models, including resistant tumors.

Results: Oral treatment with ST1926 alone caused a marginal tumor growth inhibition (<50%), but the combination with cisplatin resulted in an improved efficacy, most evident in terms of tumor growth delay without a substantial increase of toxicity. The combination therapy achieved the best effects against the HOC18 ovarian carcinoma tumor, resulting in an appreciable number of animals without evidence of disease at the end of the experiment. In contrast to the marginal effect of ST1926 alone against the subcutaneous-growing tumors, loco-regional (intraperitoneal) treatment achieved a marked increase of survival of animals with ascitic IGROV-1 tumor.

Conclusions: The present results document the efficacy of the combination of cisplatin with ST1926 and provide a rational basis for the design of novel, well-tolerated platinum-based treatment approaches in human ovarian carcinoma.

Key words: atypical retinoids, cisplatin, ovarian carcinoma, ST1926

Introduction

A group of synthetic retinoids (also known as retinoid-related molecules or atypical retinoids) has been shown to induce apoptosis by a mechanism that is independent of the retinoid receptor signaling pathway [1, 2]. Synthetic retinoids containing the adamantyl moiety represent a promising series of potentially useful agents characterized by pro-apoptotic activity in a large variety of tumor cells and antitumor activity with minimal acceptable side-effects [3–6]. We have recently reported that a novel adamantyl retinoid, ST1926, is a potent inducer of apoptosis in ovarian carcinoma cells [7]. The molecular mechanisms involved in apoptosis induction are not clearly defined. The pattern of cellular response to ST1926 supports the involvement of a genotoxic stress as a relevant aspect of the mechanism of the pro-apoptotic activity [7, 8]. Indeed, a typical feature of cellular response to ST1926 is activation of p53 and modulation of genes involved in the DNA damage response. However, both p53-dependent and p53-independent pathways appear to be implicated in apoptosis induction by ST1926 [7]. Indeed, a number of molecular events could be involved in the cellular response to adamantyl retinoids, but the critical determinants remain to be identified [9].

On the basis of its putative mechanism of action, ST1926, by inducing genotoxic stress, could sensitize tumor cells to the growth inhibition effect of clinically useful agents that cause DNA damage through different mechanisms. In the present study, we investigated the antitumor activity of ST1926 in the treatment of ovarian carcinoma models and the efficacy of its combination with cisplatin, a DNA-damaging agent, which is one of the most effective agents used in standard first-line therapy of ovarian carcinoma.

Materials and methods

Animals

The experiments were carried out on 10-week-old, female athymic Swiss nude mice (Charles River, Calco, Italy). Mice were maintained in laminar flow rooms, at constant temperature and humidity, with free access to food and water. Experiments were approved by the Ethics
Committee for Animal Experimentation of the Istituto Nazionale Tumori, Milan, according to institutional guidelines.

**drugs**

ST1926 was dissolved in a mixture of ethanol and cremophor (50 + 50%), suspended in saline (ethanol–cremophor 10% final concentration) and mixed using a magnetic stirrer. Cisplatin (Platinex, Bristol Meyers Squibb) was diluted in saline. Both drugs were administered in a volume of 10 ml/kg of body weight.

**tumor lines**

Five human ovarian tumor models were used in the study. The HOC18 tumor line was established in vivo from a pretreated patient (epirubicin, cyclophosphamide) [10]. The A2780 and IGROV-1 cell lines were derived from untreated patients [11, 12]. The A2780/DDP cell line was selected for resistance to cisplatin from the A2780 cell cultures [13]. The 1A9PTX22 cell line is a paclitaxel-resistant subline of the A2780 subclone 1A9 [14].

**subcutaneous tumor models**

For subcutaneous (s.c.) tumor systems, exponentially growing tumor cells were s.c. inoculated in nude mice. Human tumor lines were maintained by s.c. passages of tumor fragments (about 2 x 2 x 6 mm) in healthy mice. For chemotherapy experiments, tumor fragments obtained from tumor lines were used. Each group included four or five mice bearing bilateral tumors. Tumors were inoculated on day 0 and tumor growth was followed by biweekly measurements of tumor diameters with a Vernier caliper. Tumor volume (TV) was calculated according to the formula: TV (mm$^3$) = d$^2$ x D/2 where d and D are the shortest and the longest diameter, respectively. Drug treatment started at different tumor sizes. ST1926 was administered orally by gavage. A range of doses were tested following several schedules (see Tables 1 and 2). Cisplatin was delivered intravenously (i.v.) every seventh day for three times (q7d x 3). In combination studies ST1926 was given 1 h after cisplatin injection.

Drug efficacy was assessed as:

(i) Tumor volume inhibition percentage (TVI%) in drug-treated versus control mice, expressed as: TVI% = 100 – (mean TV treated/mean TV control x 100). The day of TVI evaluation is reported in the Tables 1 and 2. In the studies with drug combination the ‘expected TVI% value’ representing the TVI% due to an additive effect of the two drugs was calculated by summing TVI% induced by cisplatin + TVI% induced by ST1926 on the surviving fraction of the tumor.

(ii) Log$\text{_{10}}$ cell kill (LCK), calculated by the formula: LCK = (T - C)/3.32 x DT where T and C are the mean time (days) required for treated (T) and control (C) tumors, respectively, to reach a determined volume, and DT is the doubling time of control tumors.

Toxic effects of the drug treatment were assessed as:

(i) Body weight loss percentage (BWL%), calculated as: BWL% = 100 – (mean BW day x/mean BW day 1 x 100), where day 1 is the first day of treatment and day x is any day thereafter. The highest (max) BWL% is reported in the tables.

(ii) Lethal toxicity, assessed as deaths occurring in treated mice before the death of the first control mouse.

For statistical comparison of tumor volumes in treated vs. control mice the Student’s t-test (two-sided) was used.

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**Table 1. Activity of ST1926 and cisplatin, alone or in combination, in s.c. growing human ovarian tumor xenografts**

<table>
<thead>
<tr>
<th>Tumor (DT)a</th>
<th>Treatment (mg/kg)b</th>
<th>TVI%c</th>
<th>LCKd</th>
<th>BWL%e</th>
<th>NEDf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First day</td>
<td>Cisplatin</td>
<td>ST1926</td>
<td>Exp.</td>
<td>Obs.</td>
</tr>
<tr>
<td>A2780 (1.5)</td>
<td>2</td>
<td>30</td>
<td>40</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>40</td>
<td>40</td>
<td>0.4</td>
<td></td>
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<tr>
<td></td>
<td>3.1</td>
<td>64</td>
<td>90</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.7</td>
<td>80</td>
<td>99</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.7</td>
<td>88</td>
<td>99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2780/DDP (1.1)</td>
<td>2</td>
<td>45</td>
<td>41</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.7</td>
<td>15</td>
<td>0</td>
<td>11</td>
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</tr>
<tr>
<td></td>
<td>4.7</td>
<td>50</td>
<td>67</td>
<td>2.5</td>
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<tr>
<td>HOC18 (6.1)</td>
<td>27</td>
<td>30</td>
<td>37</td>
<td>0.4</td>
<td></td>
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<td></td>
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<td>77</td>
<td>81</td>
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<td>81</td>
<td>81</td>
<td>&gt;&gt;1.7</td>
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<td></td>
<td>4.7</td>
<td>87</td>
<td>87</td>
<td>&gt;&gt;1.7</td>
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<tr>
<td>1A9PTX22 (7.4)</td>
<td>34</td>
<td>30</td>
<td>35</td>
<td>0.3</td>
<td></td>
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<tr>
<td></td>
<td>3.1</td>
<td>38</td>
<td>0.3</td>
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<tr>
<td></td>
<td>3.1</td>
<td>60</td>
<td>67</td>
<td>0.8</td>
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<td>57</td>
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<tr>
<td></td>
<td>4.7</td>
<td>72</td>
<td>75</td>
<td>&gt;&gt;0.8</td>
<td></td>
</tr>
</tbody>
</table>

*aTumor doubling time (DT) in days.
*bCisplatin i.v. every 7th day for 3 times; ST1926, by oral route, the same day and day after the cisplatin (qd x 2/w x 3w). Only two cycles of treatment were delivered to animals bearing A2780 tumor.
*cTumor volume inhibition % = 100 – (tumor volume of treated mice over that of control mice x 100). Exp., expected in the case of an additive effect; Obs., observed experimentally.
*dGross log$\text{_{10}}$ cell kill to reach an established tumor volume (1 cm$^3$).
*eBody weight loss% induced by drug treatment; the highest value is reported.
*fNED, no evidence of disease at the end of the experiment.
intraperitoneal tumor model

The IGROV-1 ovarian carcinoma line was maintained by serial intraperitoneal (i.p.) passages in healthy mice. The tumor grows as ascites and small solid masses. IGROV-1 ascitic cells were collected and prepared as described previously [15] and 2.5 × 10⁷ cells/mouse (in 0.2 ml of saline) were i.p. injected. Mice develop hemorrhagic and diffuse carcinomatosis and eventually die by 20–35 days. Animals were inspected daily and weighed three times a week. The median day of death (median survival time, MST) was considered as the experimental end point. Experimental groups consisted of seven mice.

The drug was delivered i.p. at a dose of 10 mg/kg, daily for 5 days a week for 4 weeks (qd × 5/w × 4w), starting the day after cell injection. Drug activity was assessed as increase in life span (ILS%), i.e. the percentage increase of MST in treated (T) over control (C) mice: ((T/C) × 100) – 100. Mice alive at the end of experiment (day 88) without disease were considered ‘long-term survivors’ (LTS). For statistical comparison, curves reporting the percentage of surviving animals over time were estimated by the Kaplan-Meier product limit method and compared with the log-rank test. The test was two-sided.

tubule formation in Matrigel

Matrigel (BD Biosciences) was thawed on ice overnight at 4°C and 200 μl (at 10 mg/ml) was spread per well of a 48-well plate. The plates were polymerized for 30 min at 37°C. IGROV-1 cells were counted using trypan blue exclusion to ensure viability and plated in culture medium without serum at a density of 3 × 10⁴ viable cells/well. Cells were treated with subtoxic concentrations of ST1926. At 5, 24 and 48 h postseeding on Matrigel, tubulogenesis was observed by inverted microscope. In each condition, five randomly selected fields of view were photographed in each well. Images were captured using a Diagnostic Instruments digital camera attached to a microscope at ×4 magnification.

results

antitumor activity of ST1926

On the basis of previous studies indicating the ability of ST1926 to induce apoptosis in ovarian carcinoma cells [7], the antitumor activity of ST1926 was investigated in a panel of human ovarian carcinoma xenografts growing s.c. in female athymic mice (Tables 1 and 2). The retinoid was delivered by mouth according to various schedules. The efficacy of ST1926, given as single-agent therapy at 30 or 45 mg/kg for each administration (qd × 2/w × 2 or 3 weeks), was generally low in all the tumors investigated (tumor growth inhibition around 50%). Both doses were well tolerated (Table 1). Against the IGROV-1 tumor, various doses with daily treatment schedules were investigated without substantial improvement in the efficacy, which remained moderate (Table 2). Using the daily treatment schedule (qd × 5/w), the maximum tolerated dose was 15 mg/kg, whereas the dose of 20 mg/kg showed lethal toxicity.

In contrast to the results observed against solid tumors, ST1926, 10 mg/kg, qd × 5/w × 4 weeks, showed relevant antitumor activity in the locoregional treatment of i.p. growing IGROV-1 tumor, where it produced a significantly (P < 0.01) increased survival time over control mice, and 2 out of 7 treated animals were survivors without evidence of tumor at the end of experiment (day 88) (Table 2 and Fig. 1). efficacy of ST1926/cisplatin combination

Considering the moderate antitumor activity of ST1926 alone against solid tumor xenografts and its peculiar mechanism of action, the atypical retinoid was tested in combination with cisplatin, a DNA-damaging agent, which is one of the most effective drugs used for first-line clinical treatment of ovarian carcinoma patients. Cisplatin was delivered according to a weekly schedule (q7d × 3), which is considered as the optimal one in our experimental models, and ST1926 was administered by mouth on the same day (1 h later) and the day after each cisplatin injection (qd × 2/w). The results obtained in the tested tumor models, characterized by variable sensitivity to cisplatin, are presented in Table 1, which also reports the ‘expected value’ of TVI% of the combination, in the case of an additive effect of the two drugs. A comparison with the ‘observed value’ of TVI% indicated that the combination of cisplatin with ST1926 was able to achieve an additional antitumor effect in all of the four tumor lines investigated, even though at various levels. The tumor most sensitive to the combination was the cisplatin-sensitive A2780, because both TVI and LCK were markedly increased at both optimal (4.7 mg/kg) and suboptimal doses (3.1 mg/kg) (Fig. 2).

It is noteworthy that the effect of the combination resulted in more than additive effects even on the cisplatin-resistant A2780/DDP tumor, as documented by a high LCK value.
Against the HOC18 tumor the combination was markedly effective, because it produced a substantial increase (5/23 tumors) of complete responses (i.e. no evidence of disease at the end of the experiment) over cisplatin-treated tumors (1/10 tumors) (Fig. 4). The increase of the efficacy of the combination was less marked in the treatment of the 1A9PTX22 tumor, at least in terms of TVI% at the end of treatment (Fig. 5). However, the combination produced a substantial improvement of response in terms of tumor growth delay as compared to the treatment with cisplatin alone.

The combination of the higher dose of cisplatin with ST1926 resulted in substantially delayed tumor growth, as indicated by a marked increase in the LCK value. Concerning the toxicity of the combination, an increase in BWL was generally observed during treatment, but all mice recovered and no animals died as a result of toxicity.

**effect of ST1926 on tubule formation by IGROV-1 cells**

The substantially different effect of ST1926 against the ascitic IGROV-1 model, as compared to the same model growing as solid tumor, suggested that the growth modality and/or the
local drug concentrations may influence the tumor response. Therefore, we have tested the hypothesis that, in addition to the pro-apoptotic effects, ST1926 may exert other actions able to modulate cell behavior. Since IGROV-1 cells are able to form tubules in vitro, we investigated the effect of ST1926 during tubulogenesis (Fig. 6). Subtoxic drug concentrations (0.15 μM, i.e. around IC_{10}) caused a marked inhibition of tubule formation, already evident at 5 h of exposure.

**discussion**

Antitumor activity of a number of adamantyl retinoids, including CD437, MX3350-1 and ST1926, has been described in human tumor models of various histotypes [3]. Thus, these retinoid-related molecules represent an emerging group of promising antitumor agents endowed with remarkable apoptotic activity against leukemia cells and cells from various solid tumors [3, 16]. ST1926, one of the most promising compounds of this series, was shown to induce a high level of apoptosis in ovarian carcinoma cells at sub-micromolar concentrations [7]. Although the detailed mechanism of action of ST1926 and related molecules remains to be defined, several lines of evidence support the idea that genotoxic stress is a critical event mediating drug-induced apoptosis [7, 8, 17].

**Figure 5.** Efficacy of ST1926, cisplatin and their combination against the ovarian carcinoma paclitaxel-resistant 1A9PTX22. Arrows indicate the days of treatment with cisplatin (DDP). ST1926 was administered by mouth for two consecutive days (see Materials and methods for details). Each point refers to the mean value of 10 tumors.

**Figure 6.** Effect of a subtoxic concentration of ST1926 on tubulogenesis of IGROV-1 cells.
spite of the pro-apoptotic activity in cell cultures, the present study indicates a limited activity of ST1926 as single-agent therapy, using oral administration and a protracted treatment schedule against a panel of ovarian human tumor xenografts. In contrast, the combination with cisplatin resulted in a substantial improvement of the drug’s activity. A more than additive efficacy was achieved by the combination in both cisplatin-sensitive and cisplatin-resistant tumors, as clearly documented in A2780 and A2780/DDP models. An interesting observation of the study was the efficacy of the combination in the treatment of very responsive tumors (A2780 and HOCl18) with suboptimal doses of cisplatin (3.1 mg/kg). Whereas the treatment with each agent alone produced a marginal activity (≤50% TVI), the combination resulted in a significant increase of antitumor activity (TVI in the range of 75–90%). This observation may have obvious therapeutic implications, because the improvement of efficacy was not associated with an appreciable increase of toxicity (in contrast to the use of optimal doses of cisplatin which caused a body weight loss of around 20%).

Finally, the outstanding efficacy of ST1926 in the locoregional treatment (i.p.) of the ascitic IGROV-1 tumor could suggest the therapeutic potential of the retinoid in the control of the peritoneal effusion of ovarian carcinoma, which is a common modality of tumor growth in the natural history of the disease in patients. It is unclear whether the efficacy of this approach reflects some additional mechanisms that could influence the cell growth or migration or cell–cell interactions through inhibition of cellular adhesion. Our observations on the ability of this drug to inhibit the tubule formation in IGROV-1 cells support this hypothesis (Fig. 6). Tubulogenesis is a complex process which may be stimulated by various factors including hepatocyte growth factor (HGF) [18]. Relevant to this point is the observation that cDNA array analysis showed a down-regulation of genes encoding proteins implicated in regulatory signaling pathways (e.g. HGF and macrophage-stimulating factor) and in cell–matrix interactions (collagen, proteoglycan) (data not shown).

In conclusion, our results clearly indicate that, although ST1926 itself had modest antitumor activity against ovarian carcinoma models, it consistently sensitized ovarian tumors to cisplatin in vivo systems. The combination of platinum compounds with ST1926 might provide a rational basis for the development of more effective, well-tolerated treatment strategies for human ovarian carcinoma. Relevant to this point is the observed ability of ST1926 to sensitize ovarian tumor cells to a number of clinically useful agents, including DNA-damaging agents (e.g. DNA topoisomerase inhibitors), microtubule inhibitors (e.g. paclitaxel and vinorelbine) and the epidermal growth factor receptor inhibitor, ZD1839 [17].

acknowledgements

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