Antiangiogenic and Antitumor Activity of IDN 5390, a New Taxane Derivative

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ABSTRACT

Purpose: We previously reported that paclitaxel, a microtubule-stabilizing drug, inhibited angiogenesis, mainly by inhibiting endothelial cell motility (D. Belotti et al., Clin. Cancer Res., 2: 1843–1849, 1996). The aim of this study was to select a taxane with little cytotoxicity but with antimitotility and hence antiangiogenic activity.

Experimental Design: Different taxanes, seco derivatives, and 14-β-hydroxy-10-deacetyl baccatin III derivatives were tested for their effects on the proliferation and motility of human umbilical vein endothelial cells. The antiangiogenic and antineoplastic activities of the compound selected from this screening were further investigated in experimental models in vitro and in vivo.

Results: From the screening of different taxanes, we selected IDN 5390, a seco derivative that showed potent antimitotility activity and less cytotoxicity than paclitaxel. In comparable experimental conditions, IDN 5390 inhibited endothelial cell migration without affecting proliferation. This compound dose-dependently inhibited the capacity of human umbilical vein endothelial cells plated on Matrigel to organize into a network of cords. In vivo, IDN 5390 significantly inhibited fibroblast growth factor-2-induced angiogenesis in Matrigel implants. Daily treatment with IDN 5390 in mice bearing established lung micrometastases from the B16BL6 murine melanoma caused a reduction in the size of metastases. Finally, IDN 5390 slowed the s.c. growth of the paclitaxel-resistant human ovarian carcinoma, 1A9/PTX22, xenografted in nude mice.

Conclusions: The seco derivative IDN 5390 might represent the prototype of a new class of taxane derivatives with antiangiogenic properties.

INTRODUCTION

The importance of neovascular development, through the process known as angiogenesis, to support the growth of a tumor mass and the formation of metastases has led to great efforts to develop therapeutic tools to block this process (1). Inhibitors of angiogenesis exert antineoplastic activity in experimental models, and on the basis of promising preclinical results, many of these compounds have progressed to clinical studies (2–4).

Angiogenesis is a multistep process that can be blocked by agents affecting any one of the several events composing the whole process. The production and activity of angiogenic factors, their interaction with receptors on the surface of endothelial cells, the downstream signaling events, and the activities of endothelial cells evoked by these stimuli are all targets for antiangiogenic strategies (3).

Many crucial endothelial cell activities relevant to angiogenesis, including migration, proliferation, secretion, alignment, and formation of capillary-like structure, require a functional cytoskeleton. It has been known for some time that agents affecting the cytoskeleton, particularly tubulin-binding agents, are inhibitors of angiogenesis (5). Tubulin-binding agents, many of them initially isolated from natural sources, promote either microtubule polymerization (e.g., taxanes and epothilones) or depolymerization (e.g., colchicines and Vinca alkaloids). Despite their opposite final effects, the main activity of these compounds is likely to be kinetic stabilization of the microtubule dynamics, achieved at relatively low concentrations, with no significant changes in polymer mass (6, 7).

Microtubule-destabilizing agents, such as the colchicine derivatives combretastatin-A4 (8) and ZD6126 (9), have been developed recently as antivascular compounds because they selectively damage endothelial cells in tumor vessels, producing vascular shutdown and massive tumor necrosis.

The microtubule-stabilizing agent paclitaxel is one of several antineoplastic cytotoxic compounds with “accidental” antiangiogenic activity at subcytotoxic doses (10). Paclitaxel is a potent cytotoxic diterpene effective against a wide range of solid tumors, including breast and ovarian carcinomas. It binds to microtubules, suppressing their dynamics at substoichiometric concentrations and promoting polymerization at high concentrations (reviewed in Refs. 11, 12). Microtubule dysfunction results in mitotic block and, ultimately, in apoptosis. This activity confers to paclitaxel high cytotoxic activity on tumor cells, but is also responsible for its severe toxicity.

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The antiangiogenic activities of paclitaxel (13–15) and of the related taxane docetaxel (16) have been described in experimental models in vitro and in vivo. We previously showed that the antiangiogenic activity of paclitaxel was apparently attributable to its effects on endothelial cell motility rather than proliferation and that cell motility was inhibited in conditions in which cell proliferation was not affected (i.e., low concentrations, short exposure time; Ref. 13). This led to our research aimed at identifying paclitaxel analogues in which the two activities, inhibition of cell motility and cell proliferation, were even further apart. We therefore screened paclitaxel derivatives for their ability to inhibit endothelial cell motility, accompanied by low cytotoxicity. The screening led to the selection of the lead compound IDN 5390, whose antiangiogenic and antineoplastic activities we investigated.

MATERIALS AND METHODS

Compounds. IDN 5390 [Ref. 17; 13-(N-BOC-β-isobutyrylisoserinoyl)-10-dehydro-10-deacetyl-C-secobaccatin; $M_r$ 811; Fig. 1] and all of the paclitaxel analogues tested were obtained from INDENA SpA (Milan, Italy). For the in vitro experiments, paclitaxel and its analogues were dissolved in DMSO (1000× stock solution) and further diluted in test medium (M199–20% calf serum for the proliferation assay and DMEM-0.1% BSA for the motility assay) immediately before the assay. For in vivo administration, IDN 5390 was suspended in ethanol and Tween 80 and further diluted with saline (15:15:70, v/v/v). The dose of 120 mg/kg i.p. was chosen on the basis of preliminary dose-finding studies. This dose did not cause any signs of toxicity in the animals. For in vivo experiments, paclitaxel was administered in Cremophor EL ethanol as described (18).

Tubulin Polymerization Assay. The method described by Giannakakou et al. (19) was used. Briefly, IDN 5390 or paclitaxel (15–1500 nm) was added to 1A9 human ovarian carcinoma cells grown to confluency in 6-well plates and incubated for 6 h at 37°C. Cells were then washed twice with PBS and lysed at 37°C for 5 min in the dark with 200 μl of hypotonic buffer [1 mM MgCl₂, 2 mM EGTA, 20 mM Tris-Cl (pH 6.8), 0.5% NP40] containing protease inhibitors (Complete; Boehringer Mannheim, Mannheim, Germany). The lysates were centrifuged at 14,000 rpm for 10 min. The supernatants containing soluble (cytosolic) tubulin and the pellets containing polymerized (cytoskeletal) tubulin were subjected to Western blot analysis with monoclonal anti-β-tubulin antibody (T-4026; Sigma Chemical Co., St. Louis, MO).

Endothelial and Tumor Cells. HUVECs were isolated from umbilical cord veins and grown on 1% gelatin-coated flasks in M199 supplemented with 10% FCS, 10% newborn calf serum, 20 mM HEPES, 6 units/ml heparin, 2 mM glutamine, 50 μg/ml endothelial cell growth factor (crude extract from bovine brain), penicillin, and streptomycin. Cells were used between the third and fifth passage.

The 1A9 human ovarian carcinoma cell line and its paclitaxel-resistant variant, 1A9/PTX22 (Ref. 19; kindly supplied by A. T. Fojo, National Cancer Institute, NIH, Bethesda, MD), were cultured as described (20). Metastatic B16BL6 murine melanoma cells were cultured in Eagle’s MEM supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, and 10% FCS (21). For in vivo experiments, the tumor cells were harvested by brief exposure to 0.25% trypsin-0.02% EDTA, washed twice, and resuspended in HBSS for injection.

Motility Assay. Chemotaxis was assessed as described (13), using modified Boyden chambers and gelatin-coated polycarbonate Nucleopore filters (8 μm pore size). The supernatant of NIH-3T3 cells was used as the attractant and was added to the lower compartment of the Boyden chamber. HUVECs were detached, washed in DMEM-0.1% BSA, resuspended in the same medium at a concentration of 1 × 10⁶ cells/ml, and added to the upper compartment of the Boyden chamber. The compounds (0.01–10,000 nM) were added to the endothelial cells and incubated throughout the assay (4 h). Filters were then stained with Diff-Quik (Marz-Dade, Dudingten, Switzerland), and the migrated cells in 10 high-power fields were counted. Data are expressed as the percentage of control migration (vehicle-treated cells). The IC₅₀ (drug concentration causing 50% inhibition) was calculated from the plotted data.

Proliferation Assay. HUVECs (4 × 10⁵ cells/well) were plated in a 96-well plate in complete medium. After 24 h, the compounds (0.01–10,000 nM) were added and incubated for 4 h (as in the motility assay). Cells were then washed to remove the compounds and incubated in culture medium for an additional 3 days. They were then fixed and stained with 0.5% crystal violet in 20% methanol, rinsed with water, and air dried. The stain was eluted with a 1:1 solution of ethanol-0.1 M sodium citrate, and the absorbance at 540 nm was measured with a Multiscan MC Titertek (Flow Laboratories, Milan, Italy). Data are expressed as the percentage of control proliferation (vehicle-treated cells). The IC₅₀ was calculated from the plotted data.

Proliferation of 1A9 and I9/PTX22 cells was assessed as described above, except that 3 × 10⁶ cells/well were plated and cells were exposed to the compounds for the whole duration of the assay (72 h).

Cord Formation Assay. The ability of HUVECs to form capillary-like structures on Matrigel (Becton Dickinson, Bedford, MA) was tested. HUVECs (2 × 10⁵ cells/well in culture medium) were plated on a layer of Matrigel (10 mg/ml; 60 μl)
in 96-well plates in the presence of vehicle or the indicated concentration of IDN 5390. Pictures were taken 4 h later (when cells were aligning) and 24 h later (when cords had formed).

**Angiogenesis Assay.** The method described by Passaniti et al. (22) was used, with some modifications. Briefly, FGF-2 (300 ng/pellet) was embedded in a pellet of Matrigel (12.5 mg/ml; 0.5 ml) and injected s.c. in C57BL/6N mice (Charles River, Calco, Italy). Mice received IDN 5390 (120 mg/kg i.p.) daily for 7 days after Matrigel injection. Control mice received the same volume of vehicle. At day 7, the pellet was removed, and the hemoglobin content was measured by the Drabkin’s procedure (Drabkin reagent kit; Sigma). For histological analysis, the Matrigel pellets with the surrounding skin and tissues were fixed in 10% neutral-buffered formalin for 24 h and embedded in paraffin. Five-μm sections were stained with H&E according to standard procedures. The slides were analyzed by blind observers, and the angiogenic response was subjectively graded on a scale from 0 to 9, based on the amount of infiltrating cells and the presence of cords and erythrocyte-containing vessels, as described previously (13).

**Metastasis Assay.** C57BL/6N mice received injections of 4 × 10⁵ B16BL6 cells (in 0.05 ml) in the hind footpad. The growth of the primary tumor was measured with calipers, and when tumors reached ~250 mg, they were surgically removed. IDN 5390 (120 mg/kg) was given i.p. daily (once a day for 5 days) for 3 weeks, starting from day 3 after surgery until the end of the experiment (day 25). Mice were then sacrificed, and lungs were collected and fixed in Bouin’s solution. The number and sizes of metastases in the lungs were determined with a dissection microscope, and metastasis weight was calculated from the size of the metastases, as described previously (21).

**Human Tumor Xenograft Growth.** A suspension of 1A9-PTX22 human ovarian carcinoma cells (5 × 10⁵) was injected s.c. into the flanks of female NCr-nu/nu mice (Animal Production Colony, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD). Treatment with IDN 5390, 120 mg/kg i.p.(once a day for 5 days) for 2 weeks, started 7 days later, after randomization of mice. Tumor size was measured twice a week with calipers, and tumor weight was estimated by the formula: length × (width)²/2. Tumor weights were plotted against days after inoculation, and treatment efficacy was calculated as previously described (23). Results are expressed as optimal growth inhibition (T/C, %), defined as: (median tumor weights of treated/median tumor weights of controls) × 100, where a T/C ≤ 50% is considered active (23).

### RESULTS

**Selection of IDN 5390, a Potent Inhibitor of Endothelial Cell Motility.** To select a taxane derivative with high antiangiogenic activity but low cytotoxicity, we analyzed the effects of paclitaxel analogues on endothelial cell proliferation and motility. The analogues tested belonged to two main classes: derivatives of 14-OH-DAB and seco derivatives (Ref. 17; Table 1). All of the 14-OH-DAB derivatives inhibited endothelial cell migration but had high antiproliferative activity. The seco derivatives had little antiproliferative activity, but maintained the ability of paclitaxel to inhibit endothelial cell motility (Table 1).

#### Table 1

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<th>Compound</th>
<th>IC₅₀ (nM)</th>
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**IDN 5390 Promotes Tubulin Polymerization.** To study whether the mechanisms of action of IDN 5390 as a tubulin-binding agent were similar to those of paclitaxel, we tested its ability to promote tubulin polymerization. In cells exposed to the compound for 6 h, IDN 5390 caused a concentration-dependent increase in the fraction of polymerized tubulin and a parallel decrease in soluble tubulin (Fig. 3). Major tubulin polymerization (86%) was achieved at 1500 nM, whereas paclitaxel induced a similar effect at a lower concentration, 150 nm (Fig. 3). These findings indicate that, although less potent than paclitaxel, IDN 5390 does favor tubulin polymerization.

To investigate whether IDN 5390 shared with paclitaxel the same pattern of activity, we investigated its effect on proliferation of the 1A9 cell line and its paclitaxel-resistant variant 1A9-PTX22, which is mutated in β-tubulin (19). Although less potent than paclitaxel, IDN 5390 had a similar pattern of activity on this tumor cell system (Table 2). These findings suggest a similar mechanism of action and imply that the IDN 5390
binding site on tubulin is close, if not identical, to that of paclitaxel.

**IDN 5390 Affects Cord Formation by Endothelial Cells in Vitro.** In addition to its effects on endothelial cell motility, we tested whether IDN 5390 also prevented another endothelial cell function crucial to angiogenesis, the alignment of endothelial cells in a capillary-like structure. Endothelial cells were plated on a three-dimensional layer of Matrigel where they aligned, forming cords, which already were evident a few hours after plating. The presence of IDN 5390 in the assay caused a concentration-dependent inhibition of cord formation (Fig. 4). Although more evident 24 h after plating, when cord formation was completed, the effect of IDN 5390 was already manifest at 4 h, when endothelial cells were aligning. These findings further confirmed that IDN 5390 is able to modify endothelial cell functions at concentrations and exposure times at which cell proliferation is not affected and suggest that it might indeed prevent the process of angiogenesis.

**IDN 5390 Inhibits FGF-2-induced Angiogenesis in Vivo.** The effect of IDN 5390 on angiogenesis in vivo was evaluated in the Matrigel plug assay, where angiogenesis is induced by FGF-2 embedded in a pellet of Matrigel implanted s.c. in mice. Daily i.p. doses of IDN 5390 significantly reduced the angiogenic response induced by FGF-2 (Fig. 5). The mean hemoglobin content of FGF-2-containing pellets was significantly lower \( (P < 0.005) \) in IDN 5390-treated mice (0.012 ± 0.005 g/dl) than vehicle-treated mice (0.031 ± 0.006 g/dl; Fig. 5). Histological analysis of the Matrigel pellets confirmed the antiangiogenic activity of IDN 5390. Pellet sections from treated mice had fewer infiltrating cells, cords, and erythrocyte-containing vessels than did vehicle-treated controls (not shown). The mean histological score was 2.0 ± 0.9 for negative control pellets, 7.6 ± 0.7 for FGF-containing pellets in vehicle-treated mice (positive control), and 2.6 ± 1.1 for FGF-containing pellets in IDN 5390-treated mice \( (P < 0.001 \text{ compared with vehicle-treated mice}) \).

**IDN 5390 Impairs Spontaneous Lung Metastases by B16BL6 Melanoma.** We next evaluated whether IDN 5390 had antimetastatic activity on the murine B16BL6 melanoma transplanted in the footpads of syngeneic mice. Treatment
started 3 days after surgical removal of the primary tumor, when microscopic metastases were already implanted in the lung (24).

IDN 5390 (120 mg/kg i.p.) given daily (once a day for 5 days) for 3 weeks (days 3–7, 10–14, and 17–21) resulted in a significant reduction in the size, but not the number, of metastases in the lung (Fig. 6). No sign of toxicity was observed in the treated mice.

When IDN 5390 was tested on the primary tumor, growth was delayed throughout the duration of treatment. Tumors resumed their growth on suspension of the treatment (data not shown). These findings indicate that the compound acts by controlling tumor growth (consistent with the hypothesis of an antiangiogenic agent) rather than by eradicating existing tumor masses, as would be expected of a cytotoxic drug.

**Fig. 5** Effect of IDN 5390 on in vivo angiogenesis in the Matrigel plug assay. Matrigel containing FGF-2 (300 ng/pellet) was injected s.c. in mice. Animals were treated daily with vehicle or IDN 5390 (120 mg/kg i.p.). After 7 days, pellets were collected, and the angiogenic response was evaluated by measuring the hemoglobin content of the pellets. Data are expressed as hemoglobin content (Hb, g/dl). Horizontal bars indicate the medians. *, P ≤ 0.005 compared with vehicle-treated mice (Mann-Whitney U test).

**Fig. 6** Effect of IDN 5390 on metastasis formation by murine melanoma B16BL6 cells. B16BL6 was injected into the footpads of mice. The primary tumor was surgically removed, and treatment with IDN 5390 started 3 days later. IDN 5390 (120 mg/kg i.p.) was given (once a day for 5 days) for 3 weeks (on days 3–7, 10–14, and 17–21; n = 8–10). After 3 weeks, the number and sizes of lung metastases were recorded. Results shown are for the number of metastases per mouse (A) and the mean weight of metastasis per mouse (B). Horizontal bars indicate the medians. *, P = 0.014 (Mann-Whitney U test).

**Fig. 4** Effect of IDN 5390 on the formation of capillary-like structures. HUVECs were plated on a thick layer of Matrigel (10 mg/ml), where they rapidly aligned, forming cords. IDN 5390, at the indicated concentrations, was added to the cells 30 min after plating. Pictures were taken after 4 and 24 h (magnification, ×40).

**IDN 5390 Inhibits Growth of Paclitaxel-resistant Human Ovarian Carcinoma 1A9/PTX22 Xenograft.** The antineoplastic activity of IDN 5390 was also evaluated on the paclitaxel-resistant 1A9/PTX22 human ovarian carcinoma, implanted s.c. in nude mice. Treatment started on day 7, when tumors had a mean weight of 80 ± 30 mg. IDN 5390, 120 mg/kg i.p. daily (once a day for 5 days) for 2 weeks (days 7–11 and 14–18), significantly slowed tumor growth, with a T/C of 31% (Fig. 7). The inhibitory effect of IDN 5390 persisted as long as treatment continued, but when it stopped, on day 18, tumors started to grow at the same rate as in controls. No sign of toxicity (in terms of body weight loss) was observed. Paclitaxel administered at its maximum tolerated dose (40 mg/kg, i.v. three times every 4 days) had no activity on this tumor (data not shown), confirming that the resistance observed in vitro persisted in vivo.
validates the assumption at the basis of this study, for the cytotoxic activity (27). The seco derivatives have the integrity of the tetracyclic ring system of taxanes is required minimally cytotoxic is in agreement with previous reports that amines, which have an open C-ring at C-7 and C-8 (17), were toxic concentrations. However, our finding that the seco derivatives, which are potent inhibitors of motility, this effect occurred only at cytotoxic concentrations.

Paclitaxel, confirming previous reports (26). These compounds had high cytotoxicity, most of them being even more active than paclitaxel, confirming previous reports (26). These compounds did not meet our criteria of selection because although they were potent inhibitors of motility, this effect occurred only at cytotoxic concentrations. However, our finding that the seco derivatives, which have an open C-ring at C-7 and C-8 (17), were minimally cytotoxic is in agreement with previous reports that the integrity of the tetracyclic ring system of taxanes is required for the cytotoxic activity (27). The seco derivatives have the same ability as paclitaxel to affect cell motility. This finding validates the assumption at the basis of this study, i.e., that inhibition of motility and proliferation by taxanes are two distinct activities.

The molecular mechanism at the basis of this distinction is not known. Both paclitaxel and IDN 5390 promote microtubule polymerization and apparently bind to the same site on β-tubulin because 1A9/PTX22 cells carrying mutant β-tubulins, which confer resistance to paclitaxel (Table 2), are also less responsive to IDN 5390 in vitro. Interestingly, in a previous study we observed that 1A9/PTX22 cells, although resistant to paclitaxel in terms of inhibition of proliferation, were as responsive as the parental 1A9 cells in terms of inhibition of motility (20). This suggests a different target responsible for inhibition of motility or a different effect of taxanes on microtubule dynamics in cells during interphase or mitosis. IDN 5390 and paclitaxel might even bind to different subsets of microtubules, e.g., different β-tubulin isotypes (28) or post-translationally modified tubulin (29).

Our findings that IDN 5390 has antiangiogenic activity in vitro and in vivo suggest this compound as a prototype for a new class of antiangiogenic taxanes. However, IDN 5390 does not completely correspond to the optimal antiangiogenic compound we were searching for because it still has some cytotoxic activity, appreciable when cells are exposed to the drug for a long time. In these conditions (72-h drug exposure), IDN 5390 inhibits endothelial cell proliferation, although always less potently than paclitaxel (not shown). At present, we are searching for other seco derivatives that overcome this. Preliminary findings indicate that chemical modifications of IDN 5390 can further reduce its residual antiproliferative activity at long exposure times without changing its effects on cell motility.

The antimitotic activity of IDN 5390 was apparently sufficient to confer true antiangiogenic activity. This was supported by the finding that IDN 5390 prevented cord formation in vitro, an assay that mimics the final events during angiogenesis, when endothelial cells become organized in a three-dimensional network of capillaries. Once again, the effect occurred at concentrations that did not affect cell viability. More importantly, IDN 5390 prevented the angiogenic response induced by FGF-2 in the Matrigel plug assay in vivo, confirming its antiangiogenic activity in vivo.

An antiangiogenic compound is expected to exert a static effect on tumor growth in vivo, controlling the growth of the tumor or metastases rather than eradicating an established tumor mass. In agreement with this assumption, we found that daily treatment with IDN 5390 did control the growth of the primary tumor (1A9/PTX22 model) and of established metastases from B16BL6 melanoma. As expected, the effect required continuous administration of the compound, and as soon as treatment was suspended, tumor growth resumed.

Unlike the conventional chemotherapy approach, where maximum tolerated doses are used on an intermittent schedule to reach the end point of optimal tumor-cell kill, antiangiogenic therapy with antineoplastic chemotherapeutic drugs might require prolonged exposure to low drug concentrations. In vivo, IDN 5390 did in fact exert its antiangiogenic and antineoplastic effects when given continuously for up to 3 weeks. This schedule did not cause any evident toxicity. More thorough analysis, however, is needed to verify the pharmacokinetics of IDN 5390 and any side effects.

The in vivo activity of IDN 5390 on 1A9/PTX22 cells (resistant to both paclitaxel and IDN 5390 in vitro) suggests that the antineoplastic activity of IDN 5390 is to some extent independent of its cytotoxicity on tumor cells. The antiangiogenic nature of the antineoplastic effect of IDN 5390 needs confirmation.

In conclusion, our findings confirm the possibility of identifying well-tolerated taxanes with antiangiogenic activity. IDN 5390 is the prototype of this class of agents.
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REFERENCES

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