IDN 5390: a new concept in taxane development

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IDN 5390 is a *seco*-derivative cytostatic taxane. Originally selected for its ability to affect endothelial cell motility, the anti-angiogenic properties of IDN 5390 have been documented in experimental models, *in vivo* and *in vitro*. Preclinical studies indicate that, *in vivo*, oral IDN 5390 has a favorable bioavailability, is well tolerated and shows a significant anti-neoplastic activity on a panel of different tumor models, including paclitaxel-resistant tumors. According to its cytostatic rather than cytotoxic nature, frequent administrations of non-toxic doses have proven to be the optimal schedule for IDN 5390 treatment. Preliminary findings suggest the use of this compound in combination with conventional anti-neoplastic therapy. IDN 5390 can be considered the prototype of a new class of well-tolerated, orally available anti-angiogenic taxane derivatives with cytostatic properties. *Anti-Cancer Drugs* 14:255–258 © 2003 Lippincott Williams & Wilkins.

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Introduction: a new criterion to select taxanes

IDN 5390 was selected from a screening of paclitaxel analogs, specifically aimed at identifying anti-angiogenic taxanes able to inhibit endothelial cell motility, but characterized by scanty cytotoxicity. The rationale behind this approach derived from our previous findings of the potent anti-motility property of the microtubule stabilizing agent paclitaxel [1,2]. This was fortuitously discovered observing that trace amounts of paclitaxel strongly inhibited endothelial cell motility in the Boyden chamber assay. Motility of endothelial cells is a crucial event in the process of angiogenesis—the complex formation of a new, functional vascular network, necessary for tumor and metastasis growth [3]. The above observation therefore led us to the demonstration of the anti-angiogenic properties of paclitaxel [1]. Other studies confirmed that not only paclitaxel, but also the related taxane docetaxel, had anti-angiogenic activity and that therefore taxanes could be considered among those anti-neoplastic cytotoxic compounds endowed with 'accidental' anti-angiogenic activity [4–9].

It has been known for some time that conventional cytotoxic chemotherapeutic drugs affecting the cytoskeleton, particularly tubulin-binding agents, are inhibitors of angiogenesis, since they affect endothelial cell activities relevant to angiogenesis (including migration, proliferation, secretion, alignment and formation of capillary-like structure) [10]. However, in general, the anti-angiogenic activity is observed only at near-full cytotoxic concentrations [11,12]. At variance, different evidence suggested that the anti-angiogenic activity of taxanes occurs at low concentrations and is mainly due to an effect on endothelial cell motility rather than proliferation [1,7,9]. This is consistent with an anti-angiogenic activity of taxanes at subcytotoxic concentrations.

These results led us to a new concept for developing anti-angiogenic taxanes, based on the search for compounds in which the two activities—inhibition of cell motility and cell proliferation—are even further apart. The screening of paclitaxel analogs (*seco*-derivatives and 14-β-hydroxy-10-deacetyl baccatin III derivatives) with this criterion led to the identification of the *seco*-derivative lead compound IDN 5390 [13].

Structure of IDN 5390

IDN 5390 is a *seco*-derivative compound, characterized by an open C ring at C-7 and C-8 (Fig. 1) [14]. Structure–activity studies of taxanes have shown that the loss of integrity of the C ring, that confers rigidity to the molecular conformation, leads to a less constrained oxetane ring. This is seemingly the reason of the decreased biological activity of *seco*-derivatives [15,16]. Indeed, the reduced cytotoxicity of IDN 5390 was already known and the compound had been discarded in a screening of taxanes for higher cytotoxic activity compared to paclitaxel [17]. The structure–function relationship of IDN 5390, with specific regard to its anti-motility activity, deserves further investigation.

Anti-angiogenic activity

The anti-angiogenic activity of IDN 5390 was supported by different findings. IDN 5390 was a strong inhibitor of endothelial cell motility, as potent as paclitaxel

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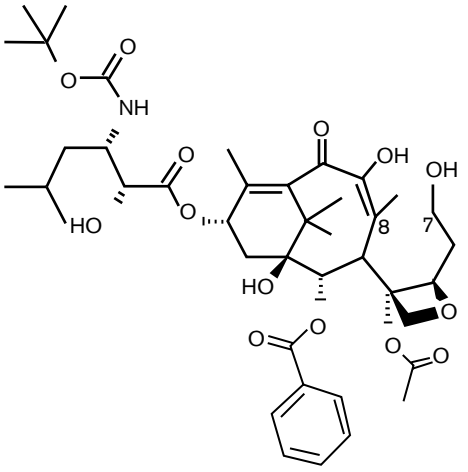
Fig. 1



Chemical structure of IDN 5390, a *seco*-derivative compound, characterized by the open C ring at C-7 and C-8.

Table 1 Effect of IDN 5390 on endothelial cell motility and proliferation

Compound	Motility [IC_{50} (nM)]	Proliferation [IC_{50} (nM)]	
	4 h	4 h	72 h
Paclitaxel	64	1188	3
IDN 5390	67	>10000	27

The effect of paclitaxel and IDN 5390 was assessed on endothelial cell proliferation (exposing the cells to the compounds for 4 or 72 h) and on motility (exposure time was 4 h). Results are expressed as IC_{50} (drug concentration, in nM, which causes 50% inhibition).

(IC_{50} = 67 nM). Inhibition of motility occurred in conditions in which cell proliferation was not affected [short exposure time (4 h) and low drug concentrations, Table 1]. IDN 5390 also prevented another cell function crucial to angiogenesis, the alignment of endothelial cells in capillary-like structures on a three-dimensional matrix [13], an assay that mimics the final events of angiogenesis, when endothelial cells become organized in a three-dimensional network of capillaries. Once again, this occurred at drug concentrations and exposure times at which cell proliferation was not affected.

In vivo, IDN 5390 inhibited FGF-2-induced angiogenesis in the Matrigel plug assay, inhibiting the infiltration of cells into the Matrigel pellet and hence preventing the formation of functional, blood-containing vascular structures [13]. Both i.p. [13] and oral [18] daily administration of the compound, at non-toxic doses, were effective in preventing the angiogenic response.

Anti-neoplastic activity

Not surprisingly, given its behavior on endothelial cells, IDN 5390 was less potent that paclitaxel also in affecting tumor cell proliferation, *in vitro*. On several cell lines and different tumor types investigated (including ovarian, breast and colon carcinoma, melanoma, and glioblastoma), IDN 5390 was 2- to 15-fold less active that paclitaxel, with IC_{50} (72 h exposure) values ranging from 5 to 61 nM [13,17–19].

Paclitaxel, as other cytotoxic compounds, is usually administered at maximum tolerated doses with extensive breaks to allow recovery from side effects. The peculiar characteristics of IDN 5390, i.e. a strong anti-motility and anti-angiogenic activity, but a poorer cytotoxicity compared to paclitaxel, indicated that the employment of this compound *in vivo* would need a specific schedule of administration. Indeed, when administered at the optimal schedule for paclitaxel (Q4 × 4) IDN 5390 failed to significantly affect the growth of a human ovarian carcinoma xenografted in nude mice [19].

The optimal schedule for treatment with cytostatic agents, such as anti-angiogenic compounds, is based on prolonged, frequent administrations [20,21]. Since these compounds do not cause permanent damage to the tumor, their continuous presence is required to maintain a constant control of vessel formation and hence of tumor growth. Indeed, prolonged and continuous administration of low doses (called *metronomic* therapy [22]) has been proposed as the preferred schedule to exploit the anti-angiogenic properties of cytotoxic anti-neoplastic agents, obtaining activity even on tumors that no longer responded to conventional treatments [8,20,21,23]. In agreement with this concept, a protracted treatment with well-tolerated doses of IDN 5390 (given daily for 5 days/week for 2–5 weeks) have been effective in reducing tumor growth of the two human ovarian carcinomas A2780/DDP [19] and A121 [18], the human breast carcinoma MX-1 [19], and the murine melanoma B16BL6 [13]. The anti-neoplastic activity was observed with i.p., s.c. and, interestingly, oral treatments with IDN 5390.

When tested, *in vitro*, on paclitaxel-resistant cell lines, the cytotoxicity of IDN 5390 was reduced compared to the paclitaxel-sensitive counterparts, with a pattern of activity analogous to paclitaxel, suggesting a similar mechanism of action. This was observed in cell systems in which drug resistance was based on different mechanisms: the ovarian carcinoma IA9/PTX22 (mutated in β-tubulin [13]), the colon carcinoma DLD1, and the two breast carcinomas MCF7/Adr and LCCS-MDR [all over-expressing P-glycoprotein (P-gp) [18]], the two ovarian carcinoma IGROV-1 (likely P-gp-positive [19]) and INT.ACP/PTX (acquired multidrug resistance [19]). Interestingly, *in vivo*, using the same resistant tumor cell lines that did not respond to paclitaxel, daily treatment with IDN 5390 caused a significant delay in the growth of three out of the four paclitaxel-unresponding models investigated [13,18,19]. This finding suggests that other mechanisms, rather than direct cytotoxicity for tumor

Table 2 Effect of IDN 5390 on metastasis formation by the murine B16BL6 melanoma

Experimental setting	Compound	Schedule of treatment	Metastasis	
			Median number (range)	Mean weight (range)
Artificial metastases	Vehicle		12 (4–33)	NE
	IDN 5390	(days −1, 0, +1)	5 (0–13)	NE
Spontaneous metastases	Vehicle		12 (3–36)	2.7 (0.01–6.72)
	IDN 5390	(Q1 × 5) × 3w	11 (2–42)	0.1 (0.07–0.76)

In the first experiment (artificial metastasis), IDN 5390 was given around the time of tumor cell injection (day 0 = day of tumor cell i.v. injection). In the second experiment (spontaneous metastasis), treatment started 3 days after surgical removal of the primary tumor, when micrometastases were already implanted in the lung. IDN 5390 was given at the dose of 120 mg/kg, i.p. NE = not evaluated.

cells, are at the basis of the anti-neoplastic activity of the drug and suggest a favorable clinical potentiality for this compound.

All the above preclinical studies reported that IDN 5390 was remarkably well tolerated *in vivo* in tumor-bearing mice. Daily doses up to 120 mg/kg could be administered without evident signs of toxicity for up to 3 weeks. Even a treatment with 90 mg/kg, given orally twice a day, 5 days/week, for up to 5 weeks was well tolerated and showed a relevant anti-neoplastic activity [19]. Toxicological studies in rodent and non-rodent species are in progress. Preliminary results indicate a good tolerability of the compound.

Anti-metastatic activity

IDN 5390 has anti-metastatic ability, as shown in two different experimental settings using the B16BL6 melanoma (Table 2). IDN 5390 prevented the implant of i.v. injected tumor cells in the lung ('artificial metastasis', Table 2). In this case, since treatment was administered at the time of tumor cell injection, we believe that the anti-motility activity of IDN 5390, also observed on B16BL6 cells, was impeding the motility of tumor cells necessary for metastatic dissemination.

Moreover, IDN 5390 was effective in blocking the growth of micrometastases. Treatment of mice after surgical removal of the primary tumor (when microscopic metastases are already implanted in the lung) resulted in a significant reduction in the size, but not the number, of metastases in the lung [13]. This indicates that the compound acts by controlling tumor growth (consistent with the hypothesis of an anti-angiogenic, cytostatic agent) rather than by eradicating existing tumor masses, as would be expected of a cytotoxic drug.

Altogether, IDN 5390 might affect metastasis through a dual mechanism: its anti-motility activity prevents tumor cell movement toward the target organ, while its anti-angiogenic activity controls the metastasis growth.

Pharmacokinetics

A high-performance liquid chromatography assay was developed to measure IDN 5390 in mouse plasma [24]. After i.v. administration of 30 mg/kg, IDN 5390 was rapidly distributed and was cleared from plasma according to a two-compartment model with a terminal half-life of 0.6 h. The clearance and volume of distribution were 4.5 l/h/kg and 5.2 l/kg respectively.

Good bioavailability of oral IDN 5390 has been shown in mice [25]. The compound is promptly absorbed with peak plasma concentration achieved within 30 min, and rapidly distributed and eliminated with a terminal half-life of 1 h even if, at the highest dose, IDN 5390 is detectable in plasma up to 4 h. The bioavailability is 40% at the doses of 60 or 90 mg/kg [25].

Conclusions and further directions

The clinical success of paclitaxel and docetaxel led to a surge of taxane analogs, mostly developed to increase the anti-tumor activity, to improve the toxicological/pharmacological profile, to overcome drug resistance or to facilitate administration/delivery [26,27]. IDN 5390 represents a new concept in developing taxanes, aimed at identifying orally available, cytostatic rather than cytotoxic compounds, more suited for a schedule of frequent, 'metronomic' administration of non-toxic doses. So far, the preclinical findings confirm the efficacy of IDN 5390, substantiating its favorable bioavailability, lack of toxicity, and its anti-angiogenic, anti-tumor and anti-metastatic activity in experimental models.

Additional studies are still needed to further validate the usefulness of this compound and to set the basis for the development of new compounds. Additional structure–function studies are expected to further improve the knowledge of IDN 5390 characteristics and to allow the selection of new compounds based on the same rationale.

The exact mechanism of action of IDN 5390 still needs to be clarified. Taxanes are known to suppress microtubule dynamics at substoichiometric concentrations and to promote polymerization at high concentrations (reviewed in [28,29]). As with the other taxanes, IDN 5390 binds to tubulin on a site close, if not identical, to that of paclitaxel [13] and, although less potent than paclitaxel, it promotes tubulin polymerization [13,19]. The high cytotoxic activity of taxanes on tumor cells, but also severe toxicity, have been related to their activity as mitotic spindle poisons, that leads to block of mitosis and

ultimately apoptosis. The significant anti-tumor activity of IDN 5390 notwithstanding its reduced cytotoxicity suggests a different mechanism of action compared to paclitaxel and the other cytotoxic taxanes. Whether this involves a different molecular target, such as different β-tubulin isotypes [30] or post-translationally modified tubulin [31], or a particular effect on microtubule dynamics in cells during interphase or mitosis, still needs to be clarified. Moreover, although our findings indicate an anti-angiogenic mechanism for the anti-neoplastic activity of IDN 5390, further studies are needed to clarify at what extent the anti-angiogenic effect of this compound is indeed the mechanism responsible for tumor growth inhibition.

Preclinical studies have demonstrated the superior anti-tumor efficacy and lower toxicity of combination treatments with conventional anti-neoplastic therapies and cytostatic compounds. Preliminary studies confirm that this is the case also for IDN 5390 [18,32]. Future studies will contribute to identify the optimal combination schedules with this new, anti-angiogenic taxane.

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