**IDN5109, a Taxane with Oral Bioavailability and Potent Antitumor Activity**

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**Abstract**

IDN5109 is a new taxane, derived from 14β-hydroxy-10-deacetylbaccatin III, selected for its lack of cross-resistance in tumor cell lines expressing the multidrug resistant phenotype. Because, unlike paclitaxel, IDN5109 is a poor substrate for P-glycoprotein, we hypothesized that IDN5109 given p.o. could improve bioavailability compared with paclitaxel. Here, we studied the p.o. and i.v. pharmacokinetics of IDN5109 together with its antitumor activity. Using a high-performance liquid chromatography method, the bioavailability of IDN5109 was determined to be 48% after oral delivery. IDN5109 given p.o. was highly active against the two human ovarian carcinoma xenografts I1A9 and HOCl8 (90–100% tumor regressions) and showed significant activity on the paclitaxel-resistant MNB-PTX1 xenograft (10% tumor regressions). The p.o. administration was as active as the i.v. route at doses reflecting the pharmacokinetic data. IDN5109 is the first taxane with good oral bioavailability and potent antitumor activity and represents a potential candidate for clinical investigation.

**Introduction**

Paclitaxel is currently used in the treatment of refractory ovarian cancer (1, 2) and exerts antitumor activity against several other tumor types such as breast cancer, melanoma, and non-small cell lung cancer (3–5). However, despite initial response rates to paclitaxel, many patients go into relapse because of the development of drug resistance after treatment (6). Another important limitation to its clinical use is poor solubility and the toxicity exerted by its vehicle, Cremophor EL (polyoxyethylated castor oil; Ref. 6). Consequently, much work has been devoted to the synthesis of analogues with less side effects, improved solubility, and enhanced antitumor activity (7–9). One of these strategies includes the identification of analogues through structure-function studies. Following this approach, new active compounds have been derived through substitutions in the 14-OH-DAB synthon (7, 8), a diterpene present in the needles of Taxus wallichiana (10). Those obtained by adding the isobutyl group at C3’ and the carbonate group at C1-C14 of the 14-OH-DAB skeleton were the more active analogues (11). IDN5109 [13-(N-boc-β-isobutylisoserinyl)-14-hydroxybaccatin-1,14-carbonate] is a paclitaxel analogue derived from this new series of 14-OH-DAB derivatives, which was selected for preclinical development because of its enhanced antiproliferative activity when compared with paclitaxel and its lack of cross-resistance in tumor cell lines expressing the typical multidrug resistant phenotype (11). Interestingly, IDN5109 proved to be 25–30 times more active than paclitaxel in two multidrug resistance-positive human cancer cell lines, MCF-7 ADR (Adriamicin-resistant) and CEM VBL (vinblastin-resistant; Ref. 11).

Paclitaxel given p.o. is poorly bioavailable. It has been suggested that the limited oral bioavailability of paclitaxel is caused by P-gp, which is abundantly present in the gastrointestinal tract (12). Accordingly, it has been observed that the oral bioavailability of paclitaxel can increase substantially with P-gp inhibitors (13, 14). The finding that IDN5109 is a poor substrate for P-gp prompted us to postulate that this taxane given p.o. could exhibit a greater bioavailability than paclitaxel.

The present study was aimed at testing this hypothesis by comparing the pharmacokinetics and the antitumor activity of IDN5109 after p.o. and i.v. administration. We show that IDN5109 possesses good oral bioavailability in nude mice, retaining significant antitumor activity against HOCl xenografts, which have a different sensitivity to paclitaxel.

**Materials and Methods**

**Animals.** Female NCr-nu/nu mice were obtained from the animal production colony of the National Cancer Institute, Frederick Cancer Research and Development Center (Frederick, MD). The mice used were 8–10 weeks of age and had a mean body weight of 23 ± 2 g. Throughout this study, nude mice were housed in filtered-air laminar flow cabinets and manipulated after aseptic procedures. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (15, 16) and international (17, 18) laws and policies.

**Ovarian Xenografts.** HOCl xenografts HOCl8 and MNB-PTX1 were established and maintained in nude mice as described previously (19, 20). The I1A9 cell line (21) is a subclone of the HOCl cell line A2780, which was established in nude mice from *in vitro* cell culture. Briefly, the cell line was grown in RPMI 1640 with 10% fetal bovine serum and 5 m *l*-glutamine. A suspension of I1A9 cells (10 × 10⁶) was injected s.c. into nude mice to obtain the corresponding xenograft. I1A9, HOCl8, and MNB-PTX1 xenografts were routinely maintained by transplanting tumor fragments into the flanks of nude mice (s.c.), where they produced progressively growing tumors.

**Drug Preparation and Administration.** IDN5109 and paclitaxel (Fig. 1) were provided by Indena (Milan, Italy) and prepared as a 30-ng/ml stock solution. Specifically, IDN5109 was prepared in a mixture containing 50% Tween 80 (polyoxyethylene-sorbitan monooleate, ICI, Cheshire, United Kingdom) and 50% dehydrated alcohol. In addition, to compare the antitumor activity of IDN5109 and paclitaxel, for i.v. injections, a formulation containing 50% Cremophor EL (BASF, Worcester, MA) and 50% dehydrated alcohol was used for both drugs. All of the stock solutions were further diluted with NaCl 0.9% (for stock solutions in Tween 80) or 5% glucose in water (for stock solutions in Cremophor EL) immediately before administration.

The maximum doses given and the optimal schedule of treatment were selected from previous dose-finding studies (data not shown). IDN5109 was given p.o. every 4 days for three injections (Q4 × 3), at the doses of 60, 90,
and 120 mg/kg. For i.v. treatments, IDN5109 and paclitaxel were administered Q4 × 3 at their MTD of 60 and 40 mg/kg, respectively. Control animals received the amount of vehicle used to prepare the highest concentration of the respective drug. Mice were weighed three times a week to evaluate drug-induced toxicity. Body weight changes were recorded and the maximum body weight loss after treatment was reported. Animals dying within 2 weeks after final drug administration were considered to have suffered toxic deaths and were excluded from further tumor response evaluation.

Pharmacokinetic Studies and Calculations. Plasma pharmacokinetic studies were carried out in female NCr-nu/nu mice receiving 60 mg/kg i.v. or 60, 90, 120 mg/kg p.o. of IDN5109.

As detailed above, IDN5109 was formulated as a 30-mg/kg stock solution (50% Tween 80 and 50% dehydrated alcohol) and diluted with NaCl 0.9% just before injection. After single i.v. and p.o. administration, blood samples were taken from four animals per time point at 5, 15, 30, 45 min and 1, 2, 4, 8, 16, and 24 h. Blood was obtained from retro-orbital plexus under diethyleter anesthesia and collected in heparinized tubes. The animals were killed by cervical dislocation. The plasma fraction was immediately separated by centrifugation at 2000 × g for 10 min at 4°C and was stored at −20°C until analysis of IDN5109 and its 7-epi-phorm, IDN5240 (Fig. 1).

IDN5109 was measured by using a recently developed high-performance liquid chromatography assay that is able to determine IDN5109 and its 7-epi-phorm with a good degree of sensitivity, precision, and accuracy (22). The method involves the addition of IDN5127 as internal standard, a totally automated solid-phase extraction on CN cartridges (Waters, Milford, MA, USA), and high-performance liquid chromatography separation on a symmetry shield column (Waters, Milford, MA), with a mobile phase of 10 mm NaH₂PO₄ (pH 5.2) and acetonitrile (47:53). The analytes were detected at 227 nm. The recovery of IDN5109 and its epi-phorm from plasma was more than 80 and 75%, respectively.

The assay was linear over a concentration range of 0.05–10 μg/ml, and all of the analytical runs performed in 3 days of the validation study had a standard correlation coefficient r > 0.995. The limit of quantitation for both of the analytes is 0.050 μg/ml, with an intra- and interday precision within 5% and an accuracy in the range of 95–107%. Pharmacokinetic parameters were calculated by using a non-linear fitting program (23). The experimental 24-h \( AUC_{v_{10}} \) and 24 h \( AUC_{p.o.} \) of IDN5109 were calculated by the trapezoidal rule, and the SD was calculated according to the method described by van Asperen et al. (24). The oral bioavailability (\( F \)) was calculated by the formula:

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F = \frac{AUC_{p.o.}}{AUC_{i.v.}} \times 100
\]

**Antitumor Activity Studies.** 1A9, HOC18, and MNB-PTX1 (as 2–3 mm tumor fragments) were implanted s.c. in the flanks of nude mice and treatments started when tumors reached approximately 150 ± 50 mg. Before distribution to the various treatments, animals were randomized on the basis of tumor size. Each treatment group consisted of 8–10 tumor-bearing nude mice. The diameters of the tumors were measured twice a week in two dimensions with a caliper, and the estimates (in g) of tumor weights were calculated as \((\text{length} \times \text{width})/2\). The end point of the experiments occurred when tumors reached a median weight of 2 ± 0.5 g or at 5 weeks after the last treatment. In the case of complete tumor regressions, the mice were observed for an additional 4 weeks to monitor regrowth of the tumors.

Tumor weights were normalized in the different groups by obtaining the RTWs, calculated by the formula: \( RTW = W/W_0 \), where \( W \) is the tumor weight at any day of measurement and \( W_0 \) is the tumor weight at the start of treatment. The median of these values (median RTW) for all of the evaluable tumors in control and treated groups was used to plot the graph and to calculate treatment efficacy (20). The T/C% ratio \( \bar{W}/[\text{median RTW of treated tumors} \times 100] \) was calculated each day that the tumors were measured, and the lowest value was considered the optimal T/C% for each group. With T/C% ≤ 50%, the treatment is considered active (20). Partial regressions correspond to greater than a 50% reduction in tumor mass. Complete regressions correspond to tumor regressions below the limit of palpation at the end of the experiment.

**Results**

**Pharmacokinetic of IDN5109 in Nude Mice.** Fig. 2A reports the plasma decay curves of IDN5109 obtained in nude mice after 60 mg/kg given p.o. or i.v. After the i.v. dose, the drug disappeared from plasma according to a two- open-compartment model with terminal half-life of 5.3 h. After p.o. administration, IDN5109 was rapidly absorbed within 1 h., then declined in the same manner as shown for the i.v. administration with a terminal half-life of 6.9 h. A comparison of three doses (60, 90, and 120 mg/kg) of IDN5109 given p.o. is shown in Fig. 2B. At all of the doses, the peak levels were achieved in 1 h, and then the drug disappeared biphasically. Table 1 reports the main pharmacokinetic parameters of IDN5109 found after the three p.o. doses and the i.v. administration. Both \( C_{\text{max}} \) and AUC values seem to be linearly related to the dose (\( R > 0.96 \)) and terminal half-life values were essentially the same among the different doses (approximately 6 h). Comparing the AUC after doses of 60 mg/kg p.o. and i.v., the bioavailability was found to be 48%. After either i.v. or p.o. administration, the 7-epi-phorm (IDN5240) was detectable up to 8 h, and its levels were approximately 10–15% of those of the parent compound. Growth inhibition effect of IDN5109 and IDN5240 was evaluated in human tumor cell lines in vitro, and no significant difference was observed between the two compounds.

**Antitumor Activity of IDN5109 after p.o. Administration.** IDN5109 given p.o. at doses of 60, 90, and 120 mg/kg against 1A9 ovarian carcinoma xenografts showed a dose-response relationship (Fig. 3). The best antitumor efficacy of IDN5109 (T/C = 1%, 7/10 partial and 2/10 complete tumor responses) was achieved at the highest dose of 120 mg/kg. At this dose, a body weight loss of 15% was reached. A good antitumor activity was found at 90 mg/kg with a T/C of 2% and 6/8 partial regressions. A minimal activity of IDN5109 was also detected at the lowest dose of 60 mg/kg, with a T/C of 22% and 1 of 9 tumor regressions.

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The antitumor activity of IDN5109 given p.o. was studied on two additional ovarian carcinoma xenografts with different sensitivity to paclitaxel, and the efficacy of the p.o. administration was compared with the treatment given i.v. Studies were performed giving IDN5109 at three dose-levels (data not shown), and results were compared at the MTD (Table 2). On the HOC18 drug-sensitive xenograft, IDN5109 given p.o. produced a significant growth inhibition with 100% of regressions (66% of total and 33% of complete). Against MNB-PTX1, a tumor poorly sensitive to paclitaxel, a remarkable antitumor efficacy was again observed after p.o. delivery of IDN5109. One complete regression (10%) was achieved at the MTD of 120 mg/kg with an optimal T/C of 20% (Table 2).

Against all of the three xenografts, the antitumor activity of IDN5109 delivered p.o. (at 120 mg/kg) closely resembled the efficacy of this compound given by i.v. delivery of IDN5109. One complete regression (10%) was achieved at the MTD of 120 mg/kg with an optimal T/C of 20% (Table 2).

Against all of the three xenografts, the antitumor activity of IDN5109 delivered p.o. (at 120 mg/kg) closely resembled the efficacy of this compound given by i.v. route at the MTD of 60 mg/kg (Table 2).

Paclitaxel, used as reference drug and given i.v. at its MTD of 40 mg/kg, produced comparable and significant antitumor response against the three ovarian tumors (Table 2).

**Discussion**

This study shows that IDN5109 given p.o. has a bioavailability of approximately 50% in mice and is active against human cancer xenografts. The hypothesis that IDN5109 given by p.o. route had a high bioavailability was based on the finding that this compound is a poor substrate for P-gp. At variance, paclitaxel is a good substrate for P-gp as documented in many studies (25, 26). The oral bioavailability of paclitaxel in mice has been reported by van Asperen et al. (14) to be less than 10%, but it increased dramatically when the oral paclitaxel was coadministered with P-gp inhibitors such as a cyclosporin A or its analogue SDZ PSC833, thus, suggesting the possibility of investigating the combination of oral taxanes with P-gp blockers at the clinical level. However, these studies showed that, in addition to a marked increase in the bioavailability of paclitaxel, P-gp inhibitors may have other mechanisms of pharmacokinetic interaction with taxanes that can modify their toxicity. In fact, SDZ PSC833 treatment caused a significant decrease of paclitaxel clearance even when paclitaxel was given i.v., presumably by reducing the rate of biliary elimination of the taxane (14). The finding reported in the present study further supports the view that P-gp is involved in the low bioavailability of paclitaxel because IDN5109, which is a taxane not pumped out of the cells by P-gp, has a much higher bioavailability than paclitaxel. With this in mind, the administration p.o. of IDN5109 seems to be the preferred alternative to the combination of conventional taxanes with P-gp inhibitors.

The data on the antitumor activity of IDN5109 are in line with the pharmacokinetic findings. After administration p.o., IDN5109 was highly active against the two paclitaxel-responsive 1A9 and HOC18 xenografts, causing complete tumor regressions. IDN5109 showed significant activity also on MNB-PTX1, a tumor poorly responsive to paclitaxel (14). The antitumor activity of IDN5109 administered p.o. against 1A9 s.c. xenograft. Nude mice were transplanted s.c. with 1A9 fragments, and treatments started 11 days after tumor transplantation (n = 7). IDN5109 was given p.o. every 4 days for a total of three injections (Q4 × 3). , vehicle; , IDN5109 60 mg/kg; , IDN5109 90 mg/kg; , IDN5109 120 mg/kg.

**Table 1** Main pharmacokinetic parameters of IDN 5109 in nude mice

<table>
<thead>
<tr>
<th>Injected dose (mg/kg)</th>
<th>Route of injection</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml ± SD)</th>
<th>Terminal half-life (h)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>AUC&lt;sup&gt;c&lt;/sup&gt; µg/ml · h&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>60 i.v.</td>
<td>107.3 ± 6.8</td>
<td>5.3</td>
<td>74 ± 9 (77)</td>
<td></td>
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<tr>
<td>60 p.o.</td>
<td>7.3 ± 0.7</td>
<td>6.9</td>
<td>35 ± 7 (37)</td>
<td></td>
</tr>
<tr>
<td>90 p.o.</td>
<td>9.35 ± 1.52</td>
<td>6.9</td>
<td>41 ± 5 (44)</td>
<td></td>
</tr>
<tr>
<td>120 p.o.</td>
<td>12.62 ± 1.94</td>
<td>7.2</td>
<td>53 ± 8 (57)</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> Pharmacokinetic parameters were calculated by using a non-linear fitting program.
<sup>b</sup> Single injection.
<sup>c</sup> AUC values were calculated for 24 h. In parentheses, AUC ∞.
<sup>d</sup> Data are the mean of 4 animals ± SD.

**Fig. 2.** IDN5109 levels in nude mice. A, plasma concentration versus time curves of IDN5109 in nude mice after i.v. ( - ) or p.o. ( - ) administration of 60 mg/kg. B, plasma concentration versus time curves of IDN5109 in nude mice after p.o. administration of 60 ( - ), 90 ( - - ), and 120 mg/kg ( - ). Data are the mean of four mice per time point ± SD. The absence of bars indicates that the SD is smaller than the size of the symbol.

**Fig. 3.** Antitumor effect of IDN5109 administered p.o. against 1A9 s.c. xenograft. Nude mice were transplanted s.c. with 1A9 fragments, and treatments started 11 days after tumor transplantation (n = 7). IDN5109 was given p.o. every 4 days for a total of three injections (Q4 × 3). , vehicle; , IDN5109 60 mg/kg; , IDN5109 90 mg/kg; , IDN5109 120 mg/kg.
that is highly active in ovarian carcinoma. Given its good bioavailability and efficacy after p.o. administration, the development of oral IDN5109 is highly recommended.

Acknowledgments

We thank Maria Pirocchi for style editing.

References


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