Pegylated Liposomal Doxorubicin and Vinorelbine in Recurrent Ovarian Carcinoma: A Pharmacokinetic Study on Alternate Administration Sequences

LUIGI CATTEL¹, ROBERTO PASSERA¹, DYONISSIOS KATSAROS², MAURIZIO MEDAIL¹, PAOLA MILLA¹ and ANNA MARIA FERRERO³

¹Dipartimento di Scienza e Tecnologia del Farmaco, ²Dipartimento di Ostetricia e Ginecologia and ³Divisione di Ostetricia e Ginecologia, Unità di Ginecologia Oncologica, Ospedale Mauriziano, Università di Torino, Italy

Abstract. Background: This multicenter pharmacokinetic study evaluated the pharmacokinetics and toxicity of the combination of pegylated liposomal doxorubicin (PLD) and vinorelbine (VNR) in patients with recurrent ovarian cancer, when administered in opposing sequences. Patients and Methods: Eighteen heavily pre-treated ovarian cancer patients received treatment with PLD 30 mg/m² and VNR 30 mg/m² every 3 weeks for 6 cycles, 9 being given the PLD-VNR sequence vs. 9 the VNR-PLD sequence. Results: The VNR AUCtot and plasma levels were considerably higher with the PLD-VNR sequence and VNR clearance, clearly related to K.el, was about half that occurring with VNR-PLD. Toxicity was generally mild and reversible. In both arms, a sound correlation was found between VNR AUCtot and absolute neutrophil count decrease. A possible correlation between hematological toxicity and the 2 opposing administration sequences was also shown. Conclusion: The higher VNR AUCtot and plasma levels during the elimination phase with the PLD-VNR sequence and VNR clearance, clearly related to K.el, was about half that occurring with VNR-PLD. Toxicity was generally mild and reversible. In both arms, a sound correlation was found between VNR AUCtot and absolute neutrophil count decrease. A possible correlation between hematological toxicity and the 2 opposing administration sequences was also shown. Conclusion: The higher VNR AUCtot and plasma levels during the elimination phase with the PLD-VNR sequence may be related to a P-gp membrane glycoprotein inhibition by PLD vesicles which, in turn, may influence the plasma level of the associated VNR. PLD-VNR, producing a higher plasma level and very mild toxicity, may be considered the preferred sequence.

Pegylated liposomal doxorubicin (PLD) is a preparation of doxorubicin hydrochloride acid that is encapsulated in pegylated liposomes to prevent recognition by the immune system, thus increasing its serum half-life and reducing its toxicity (1). Pegylated liposomes, in comparison to non-pegylated ones, have a much longer half-life, due to their coating of methoxypolyethylene glycol polymers, which avoids liposomal detection and destruction by the reticuloendothelial system. Moreover, the very small diameter of pegylated liposomes, together with the so-called EPR effect (enhanced permeability and retention), favors a higher doxorubicin concentration in the tumor tissue (2).

PLD pharmacokinetics (pk) is quite different from that of conventional doxorubicin; both the volume of distribution and plasma clearance are dramatically lower, resulting in 1000-fold lesser AUCtot compared to the standard formulation. These pk properties and more permeable blood vessels in tumors allow PLD to preferentially accumulate in tumor tissues.

Pre-clinical studies have reported the superiority of liposomal over free doxorubicin in ovarian cancer (3) and activity against several other tumors (4). Many phase II clinical trials of PLD, employed as a single agent in advanced epithelial ovarian cancer, have shown response rates ranging from 15 to 31% (5, 6), whereas combinations of PLD with other drugs in the second-line treatment of ovarian cancer have shown promising results (7, 8).

Vinorelbine (5’-nor-anhydrovinblastine, VNR) is a semi-synthetic anticancer agent of the Vinca alkaloid group, with reduced neurotoxicity compared to its congeners (9). Although it is derived from vinblastine, VNR differs structurally due to the altered catharanthine moiety, which results in an 8-membered ring versus the 9-membered ring characteristic of Vinca alkaloids. This structural modification on the catharanthine moiety gives VNR highly specific antimitotic properties, as well as enhancing its lipophilicity (10).

After i.v. infusion, VNR is characterized by rapid plasma clearance, a large volume of distribution and a long terminal half-life. Seventy to 80% of the administered dose is eliminated by biliary excretion; only 11% is excreted by the
renal route, the majority being eliminated through fecal excretion. VNR metabolism is mainly hepatic, involving CYP450 3A4 isoforms; one main metabolite, 4-O-deacetyl-VNR, and two minor ones, 20'-hydroxy-VNR and VNR-6'-oxide, have been found.

Vinorelbine has a better toxicity profile than vincristine (11) and promising activity as a single agent (12). Many phase I and II studies have been conducted to assess the feasibility and efficacy of combinations of vinorelbine and other cytotoxic agents in combating recurrent ovarian cancer (13, 14). In particular, the combination of vinorelbine and docetaxel has recently been shown to be effective, with manageable toxicity, against platinum-resistant relapsed ovarian cancer (15).

On the basis of the reported activities of PLD and VNR in monotherapy, it appeared interesting to study a combination of PLD and VNR in advanced epithelial ovarian cancer treatment. Indeed, a recent dose-finding study evaluated the combination of PLD and VNR for the treatment of refractory or resistant epithelial ovarian cancer (16); however, the study examined neither pk data nor any possible pk interaction between the 2 drugs. On the contrary, both PLD and VNR have been widely studied for their clinical pk profile in monotherapy (17, 18). Based on these data, we conducted a phase II multicenter pk trial of pegylated liposomal doxorubicin and vinorelbine in heavily pretreated ovarian cancer patients, in order to compare any pk interaction between the 2 drugs administered following alternate sequences: PLD-VNR and VNR-PLD. The relationship between pk data and toxicity in the 2 opposing schedules were also studied. A clinical overview of this study, with a preliminary pk report on this new anticancer regimen, has been recently published by our group (19).

Patients and Methods

Patient characteristics. This prospective multicenter pk phase II study enrolled 18 patients. The eligibility criteria included: a) age between 18 and 70 years; b) platinum- and taxane-pretreated epithelial ovarian cancer; c) progressive disease with measurable/evaluable lesions, documented through imaging procedures; d) life expectancy >3 months; e) adequate bone marrow, renal and hepatic functions (creatinine, serum bilirubin, AST/ALT less than twice the normal upper limit); f) normal cardiac function, evaluated by ECG and echocardiography (with ejection fraction >52); g) Eastern Cooperative Oncology Group (ECOG) Performance Status ≤2; h) written informed consent.

The exclusion criteria included: a) patients previously treated with anthracyclines (maximum dose >360 mg/m² for doxorubicin); b) previous or concurrent neoplasm other than the ovarian one (with the exception of cutaneous basalioma and cervical intraepithelial neoplasia); c) severe cardiac dysfunction or uncontrolled hypertension.

The median number of previous chemotherapy regimens was 2, with a range from 1 to 6. Six patients (3 in each group) had received an anthracycline-based regimen, while no subject had previously been given any Vinca alkaloid. No patients received abdominal radiation therapy or were subjected to liver resections before entering the pharmacokinetic study. Six patients (3 in each group) had metastatic liver disease. The study was conducted in accordance with the Tokyo-Helsinki Declaration and Good Clinical Practices, and was approved by the local Ethics Committees.

Treatment plan. All 18 patients were given the 2 drugs in sequence: 9 received PLD followed by VNR (A) and 9 received VNR followed by PLD (B). They were randomly allocated to the 2 experimental groups through a centralized procedure at the Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Italy, using a random numbers electronic table. In sequence A, on day 1 PLD was given first at 30 mg/m² in a 30-min i.v. infusion followed, after a 30-min interval, by VNR at 30 mg/m² in a 15-min i.v. infusion. In sequence B, on day 1 VNR was given first at 30 mg/m² in a 15-min i.v. infusion followed, after a 30-min interval, by PLD at 30 mg/m² in a 30-min i.v. infusion. Both treatments were repeated every 3 weeks for 6 cycles until progression, unacceptable toxicity or patient refusal intervened. Standard antiemetic treatment was given to all patients, preventing palmar-plantar erythrodysesthesia (PPE) by oral administration of corticosteroids (during the first week after infusion) and pyridoxine (100 mg/day, during intervals between cycles). Apart from them, concomitant CYP3A inhibitors/inductors were not allowed. Hematological toxicity was evaluated by a complete blood count every week, while non-hematological toxicity was assessed before each cycle.

Blood samples. The patients were examined during their first cycle; only 1 patient was given both alternated sequences during the first and second courses, as an internal control. Blood samples were collected in tubes from a large vein in the arm not receiving the drug infusion.

For VNR, 7 blood samples were drawn for both sequences A and B (immediately before the VNR infusion, at the end of the VNR infusion, 5, 20, 60, 90 and 120 min after the end of the VNR infusion). After 120 min, the VNR plasma levels quickly reached the limits of analytical detection (20-40 ng/ml).

For PLD, only 1 sample was collected for both sequences, at the end of the PLD infusion; this was because our main interest lay in detecting any PLD interactions vs. VNR pk behavior, using PLD C_end of infusion as a predictor of C_max.

Materials and equipment. VNR (Navelbine®) was kindly provided by Pierre Fabre, Italy, while PLD (Caelix®) was from Schering Plough, Italy. The HPLC system for VNR consisted of a Shimadzu LC-10ADvp pump and a Shimadzu SPD-10Avp UV spectrometer; the analytical column was a Symmetry C18 Waters (250x4.6 mm, i.d. 5 µm), UV detection at 285 nm and isocratic run. The mobile phase consisted of a mixture CH₃CN : phosphate buffer solution (PBS) 25 mM pH 6.4 (15:85 v/v) and CH₃CN : PBS 100 mM pH 10 (50:50 v/v), containing 0.1 g/l sodium dodecyl sulphate. The HPLC system for PLD consisted of a Merck-Hitachi L-6200 pump and a Shimadzu RF551 fluorescence detector; the analytical column was a LiChrosorb C18 Merck (250x4.6 mm, i.d. 10 µm), with fluorescence detection (475 nm excitation and 580 nm emission). A linear mobile phase gradient, consisting of 2 mixtures CH₃CN : phosphate buffer solution (PBS) 100 mM pH 2 (15:85 v/v) and CH₃CN : PBS 100 mM pH 2 (50:50 v/v), was used.
Analytical procedures. The VNR plasma concentrations were measured with a modified known procedure (17). To 1 ml plasma samples, 1 ml 66 mM PBS pH 7 and 5 ml diethyl ether were added. After vortex-mixing and centrifugation at 3,000 rpm for 10 min, the samples were placed in a freezer at −20°C until the water phase froze. The organic phase was then transferred into screw-cap tubes and dried under a nitrogen stream at 25°C. The residue was dissolved in 800 µl of the HPLC mobile phase, vortex-mixed and filtered through Millex SLCR filters; 200 µl were injected into the HPLC system. The linear gradient started from 100% first mixture and reached 100% second mixture in 18 min, at 1 ml/min flow; the PLD retention time was about 11 min.

A modified plasma extraction procedure for PLD was used (18). Five ml plasma samples, cooled at 4°C, were centrifuged at 3,000 rpm for 10 min, then stored at −70°C. A 500-µl plasma aliquot was fortified with 100 µl CH3CH2OH and vortex-mixed. The further addition of 50 µl Triton X-100 3% was required to break the liposome vesicles. After a second vortex-mixing procedure, 50 µl 5-sulphosalycilic acid 65% were added to precipitate both liposomal components and plasma proteins, which were removed by a 10-min 20,000 rpm centrifugation. After discarding the pellet, the resulting solution was fortified with 15 ml PBS 1.8 M, diluted 1:20 with HPLC mobile phase and filtered through Millex SLCR filters; 200 µl were injected into the HPLC system. The linear gradient started from 100% first mixture and reached 100% second mixture in 18 min, at 1 ml/min flow; the PLD retention time was about 11 min.

Pharmacokinetic and statistical analysis. The pk study entailed a typical model-free approach, non-compartmental analysis (NCA). The purpose of NCA is to provide an estimate of the kinetic parameters of a drug, like AUC or MRT, based on statistical moment theory. These parameters are determined by a numerical integration procedure, such as the trapezoidal rule; the only assumption is that the terminal elimination phase of the drug can be described by a monoexponential equation, following a first order kinetics. NCA was performed using Kinetica 2000 4.1.1 software (InnaPhase Corp., USA), with the following parameters: Cmax (maximum concentration), AUCtot (total area under concentration-time curve), Kel (elimination rate constant), t1/2 (elimination half-life), MRT (mean residence time), Cltot (total clearance), Vz (volume of distribution of the late elimination phase) and Vss (volume of distribution at the steady state).

Results

Pharmacokinetics. The VNR plasma concentration profiles (Cmax and AUCtot) in the PLD-VNR sequence were clearly higher than those found in the opposing sequence (VNR-PLD) (Table I, Figure 1); the difference was statistically significant for AUCtot (A 648.43 vs. B 294.67 ng*h/ml, p=0.0003), while Cmax did not achieve a statistically significant difference (A 1479.89 vs. B 983.86 ng/ml, p=0.1135), even increasing by 50% in the PLD-VNR sequence. Cltot showed similar behavior (A 77.84 vs. B 155.44 l/h, p<0.0001), which may be due to different Kel values rather than altered distribution volumes (Vz and Vss). In order to check whether the pk interaction was due to the opposing administration sequence, 1 patient was given sequence A in cycle 1 and sequence B in cycle 2 (Figure 2): both the concentration-time profile and the pk parameters were identical to those of all other patients. We also analyzed PLD plasma values at the end of the i.v. infusion, roughly representative of PLD Cmax: in sequence A, this value was not significantly higher than in sequence B (A 22.87 vs. B 16.82 mg/l, p=0.1139). The VNR and PLD pk parameters were in good agreement with those previously reported in monotherapy trials (20,21).

### Table I. VNR and PLD pharmacokinetic results (median + range).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PLD-VNR sequence</th>
<th>VNR-PLD sequence</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax ng/ml</td>
<td>1479.89 (360-5274)</td>
<td>983.86 (245-1645)</td>
<td>0.1135</td>
</tr>
<tr>
<td>AUCtot ng*h/ml</td>
<td>648.43 (357-1087)</td>
<td>294.67 (170-438)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Kel 1/h</td>
<td>0.37 (0.27-1.22)</td>
<td>0.73 (0.43-8.54)</td>
<td>0.0315</td>
</tr>
<tr>
<td>t1/2 h</td>
<td>1.87 (0.57-2.55)</td>
<td>0.95 (0.08-1.62)</td>
<td>0.0315</td>
</tr>
<tr>
<td>MRT h</td>
<td>1.27 (0.52-3.50)</td>
<td>0.84 (0.18-1.37)</td>
<td>0.0625</td>
</tr>
<tr>
<td>Cltot l/h</td>
<td>77.84 (40-112)</td>
<td>155.44 (118-247)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vz l</td>
<td>173.15 (68-314)</td>
<td>181.64 (18-371)</td>
<td>0.4894</td>
</tr>
<tr>
<td>Vss l</td>
<td>96.97 (25-189)</td>
<td>142.70 (27-179)</td>
<td>0.8633</td>
</tr>
<tr>
<td>Cend of infusion mg/l</td>
<td>22.87 (14-29)</td>
<td>16.82 (12-25)</td>
<td>0.1139</td>
</tr>
</tbody>
</table>

* Mann-Whitney non-parametric test

Cmax (maximum concentration), AUCtot (total area under concentration-time curve), Kel (elimination rate constant), t1/2 (elimination half-life), MRT (mean residence time), Cltot (total clearance), Vz (volume of distribution of the late elimination phase) and Vss (volume of distribution at the steady state).
Toxicity. The expected toxicity for this combination included alopecia, fatigue, nausea, anemia, leucopoenia and PPE (16). The VNR specific toxicity was generally mild: neutropenia being G3 in only 3 patients (2 in PLD-VNR and 1 in VNR-PLD). On the other hand PPE, typical of PLD, occurred at G3-4 in only 2 patients; PLD induced lower incidence and toxicity rates compared with data reported by Gordon et al., when PLD was administered as a single agent (7). The serum bilirubin and AST/ALT levels did not differ between the 2 groups ($p > 0.05$). The possible correlation between hematological toxicity and administration sequence was also studied. The absolute neutrophil count/µl (ANC) after 1, 2 and 3 weeks was clearly different, even without reaching statistical significance ($p = 0.80, 0.27, 0.93$). As shown in Figure 3, the neutrophil nadir differed in the opposing sequences, being reached after 1 week for VNR-PLD (similar to VNR monotherapy) and after 2 weeks for PLD-VNR (as in PLD monotherapy). Moreover, in both arms a sound correlation was found between VNR $AUC_{tot}$ and ANC decrease ($A r^2 = 0.929$ and $B r^2 = 0.878$).

In our clinical study (19), the overall response rate (ORR=CR+PR) was 43% (0%+43%) after 3 cycles, with SD 27% and PD 30%. The efficacy data are quite interesting, in the light of the low toxicity profile and considering that VNR administration was more delayed than in the monotherapy regimens.

Discussion

This study determined that both VNR $C_{max}$ and $AUC_{tot}$ were considerably higher when PLD was administered before rather than after VNR. These VNR pk data are in good agreement with the remarkable decrease in VNR $Cl_{tot}$ in the same sequence, depending on $K_{el}$ alteration. The pk behavior may suggest that PLD interferes with VNR during its elimination phase (mainly biliary). It is known that Vinca alkaloids, anthracyclines and taxanes are substrates of P-gp membrane glycoprotein; many in vitro and in vivo studies have shown that P-gp plays a significant role in the biliary excretion of P-gp substrates. P-gp is highly expressed on canalicular membranes of hepatocytes. It has also been suggested that inhibition of P-gp could modulate and enhance the plasma levels of associated P-gp substrates. Gianni et al. first demonstrated a doxorubicin-paclitaxel interaction, due to P-gp inhibition by Cremophor EL (paclitaxel excipient) (22). Similarly, our group has shown that, when docetaxel is associated with epirubicin, some minor pk interactions occur, possibly due to the P-gp inhibition by Tween 80 (docetaxel excipient) (23). It is difficult to suggest a direct role of free doxorubicin to explain the VNR pk interactions.
alterations, because the drug remains encapsulated within the liposomes for a long time: after PLD i.v. infusion, very little free doxorubicin can be found in the blood stream, most of the drug remaining encapsulated (1). Consequently, it is unlikely that PLD pharmacokinetically interferes with any other associated anticancer drug. A recent investigation has shown that the reduced toxicity and enhanced therapeutic efficacy shown by liposome-associated drugs may be due to the inhibition of P-gp membrane glycoprotein by the phospholipid vesicles (24). Similarly, recent researches employing LY-335979 (P-gp inhibitor) and VNR reported a marked decrease in VNR plasma clearance: the inhibition of P-gp in the bile canalici by this compound most probably impedes VNR biliary excretion (25). Our final suggestion is that PLD-phospholipid vesicles could inhibit P-gp activity during the VNR elimination phase, resulting in plasma accumulation of other co-administered P-gp substrate drugs.

In an attempt to elucidate any correlation between VNR pk parameters and clinical toxicity, an inverse linear relationship was found between ANC and VNR AUC_cTot. Moreover, the hematological toxicity evaluated as ANC differed markedly in the opposing sequences, being reached after 1 week for VNR-PLD (as in VNR monotherapy) and after 2 weeks for PLD-VNR (as in PLD monotherapy). Nevertheless, due to the limited number of patients, the difference between the 2 arms did not achieve statistical significance. The final results of this research will be of value in investigating the relationships between pk/toxicological behavior and clinical efficacy. However, the PLD-VNR sequence, having higher VNR plasma levels and a safer toxicity profile, should be considered the preferred administration sequence.

References


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