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Plasma and cerebrospinal fluid pharmacokinetics of vincristine and vincristine sulfate liposomes injection (VSLI, marqibo®) after intravenous administration in Non-human primates

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Summary

Purpose—Vincristine sulfate liposomes injection (VSLI, Marqibo®) is an FDA approved encapsulated preparation of standard vincristine in sphingomyelin/cholesterol liposomes. Clinical pharmacokinetics show VSLI to be a long-circulating, slow release formulation that is confined to plasma, and prior data on cerebrospinal fluid (CSF) pharmacokinetics are lacking. We report our results comparing CSF and plasma pharmacokinetic parameters of intravenous aqueous vincristine to intravenous VSLI using an established non-human primate (NHP) model.

Methods—Three adult male rhesus monkeys (*Macaca mulatta*) were administered 0.1 mg/kg (1.2 mg/m² human-equivalent dose) of vincristine or VSLI in a crossover pharmacokinetic study. Serial paired blood and CSF samples were obtained before infusion, at the end of infusion (EOI) and at various time points thereafter.

Results—In contrast to standard vincristine, which had a multi-exponential plasma disappearance curve with a median initial (EOI to 30 min post-infusion) half-life ($T_{1/2}$) of 4.8 min (range, 4.4–5.0 min) and terminal $T_{1/2}$ of 24.3 h, a near-monoexponential curve with a median $T_{1/2}$ of 17.9 h (range, 13.9–21.5 h) hours was calculated with VSLI. The ratios $Cl_{VCR}:Cl_{VSLI}$ for the individual NHP were 300, 463 and 477. Vincristine was not detected in any CSF sample after administration of either formulation. **Conclusions** In three animals, each serving as their own control, we demonstrate that the pharmacokinetic profile of VSLI shows markedly prolonged clearance (approximately 400-fold lower) of total vincristine in comparison to the standard aqueous formulation, enhancing our understanding of VSLI pharmacokinetics. Several clinical trials incorporating VSLI as substitution for standard vincristine are in progress.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Keywords

Non-human primate model; Cerebrospinal fluid; Pharmacokinetics; Vincristine sulfate liposomes injection

Introduction

Vincristine sulfate is a vinca alkaloid derived from the periwinkle plant (*Vinca rosea*). Vincristine sulfate has a broad antitumor spectrum and is an important component of combination chemotherapy regimens in the treatment of acute lymphoblastic leukemias (ALL), Hodgkin's and non-Hodgkin's lymphomas (NHL), sarcomas, CNS tumors and various other malignancies. Limitations to vincristine therapy include treatment related neurotoxicity, due to which individual doses of vincristine are limited (e.g., a standard vincristine dose is 1.5 mg/m²/dose, capped to a maximum of 2 mg/dose), to reduce the risk of severe neurotoxicity. Prior attempts at dose intensification of vincristine in with the goal to improve efficacy have not been successful due to excessive peripheral neuropathy [1].

Vincristine sulfate liposomes injection (VSLI/Marqibo®) is a novel formulation of vincristine encapsulated in sphingomyelin/cholesterol liposomes specifically designed to facilitate the loading and retention of standard vincristine to enhance the therapeutic index of vincristine prolonging the circulation time of encapsulated vincristine. Both preclinical [2–4] and clinical data suggest safety and efficacy of VSLI. VSLI was recently granted FDA accelerated approval for the treatment of adults with Philadelphia chromosome negative acute lymphoblastic leukemia (ALL) in second relapse, based on an impressive 20 % complete remission rate in adults with relapsed/refractory disease using single agent therapy [5, 6].

Clinical pharmacokinetics studies have shown VSLI to be a long-circulating, slow release formulation that is confined to plasma [7, 8]. A delay of 3 to 12 h in vincristine clearance from plasma is observed in adults following VSLI administration, resulting in total vincristine levels that remain relatively constant before declining with time. This delay phase contributes significantly to the plasma exposure (i.e., area under the concentration x time curve of total vincristine) after VSLI administration which, when compared to published data, is increased compared to that following vincristine administered as vincristine sulfate [7, 8]. Additionally, limited comparative pharmacokinetic parameters of aqueous vincristine and VSLI have been studied in rat, mouse and dog models, [7, 9] however, these formulations have not been directly compared in non-human primates (NHP). Additionally, there have been no known studies of central nervous system pharmacokinetics of liposomal encapsulated vincristine.

Our center has continuously maintained a NHP model that has been highly predictive of human plasma and cerebrospinal fluid (CSF) pharmacokinetics of a number of anticancer and antiretroviral drugs. Many of these animals have a chronically indwelling Pudenz 4th ventricular catheter attached to a subcutaneous Ommaya reservoir, which allows for sampling of CSF at multiple time points in unanesthetized animals [10]. Utilizing the NHP model to evaluate pharmacokinetic parameters of these two formulations of vincristine

sulfate, we performed the first known head-to-head comparative pharmacokinetic study of standard vincristine versus VSLI in primates. We report our results on the plasma and CSF pharmacokinetics of intravenous aqueous vincristine compared to intravenous VSLI.

Materials and methods

Animals

Three adult male rhesus monkeys (*Macaca mulatta*) ranging in weight from 11 to 14 kg were used in this study, with each animal serving as his own control. The animals were SRV/SIV and Herpes B virus negative. The National Cancer Institute (NCI) Institutional Animal Care and Use Committee approved this study. All animals were socially housed in groups, fed LabDiet Monkey Chow #5038 with water ad libitum and cared for in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals [11]. Animals were restrained for serial sampling via the pole and collar system. CSF samples were obtained from a chronically indwelling Pudenz silicon catheter attached to a subcutaneously implanted Ommaya reservoir (Fig. 1) [12]. The reservoir is pumped 4 times before and after each CSF sample collection to ensure adequate mixing with ventricular CSF. Blood samples were obtained from a central venous access port, which was different from the site of drug infusion.

Drug

Vincristine sulfate liposome injection (VSLI, 0.16 mg/mL) was provided by Talon Therapeutics Inc., (subsequently Spectrum Pharmaceuticals, Inc., Irvine, CA) as a single-use kit consisting of three components and was formulated according to package directions (www.marqibo.com/hcp/prep.php). Clinical grade standard aqueous preparation vincristine sulfate was purchased through the Veterinary Resource Branch, National Institutes of Health (NIH).

Experiments

All animals were administered 0.1 mg/kg (1.2 mg/m² human-equivalent dose) of standard vincristine or VSLI over 15 min on separate occasions, in a sequence that was randomly assigned and spaced apart by a minimum of 2 weeks. Serial paired blood (1 mL/sample) and CSF (0.3 mL/sample) samples were obtained before infusion, at the end of infusion (EOI) and 5, 15 and 30 min and 1, 2, 3, 4, 6, 8, 24, 48, 72 and 96 h following the completion of the infusion. Plasma was immediately separated by centrifugation. Plasma and CSF samples were stored at -80 °C until analysis.

Sample Analysis

Drug concentrations were quantified using validated HPLC/tandem mass spectrometry assays. Total plasma vincristine (liposomal encapsulated, non-encapsulated, and protein bound) was measured following extraction of drug from plasma samples. Briefly, 5 µl of normal plasma containing 10 µg/ml of vinblastine was added to 45 µl of plasma collected from NHP at prescribed times. To this mixture, 450 µl of methanol was added, the preparation was vortexed for 5 min, centrifuged at 10,000 rcf and methanolic supernatant was removed for analysis. Vincristine (retention time 1.2 min) and vinblastine (retention

time 1.4 min) were separated on a Waters 5 μ m 2.1 $\times$ 50mm XBridge C18 column (Waters Corp, Milford MA), using an isocratic mobile phase consisting of 1 mM ammonium acetate, 0.1 % formic acid, 10 % acetonitrile, 40 % methanol at 0.2 mL/min using a Shimadzu HPLC system consisting of 2 LC-20 AD pumps, SIL-20 AC autosampler, CTO-20 AC column heater and CBM-20A controller (Shimadzu Corp., Kyoto, Japan). Vincristine and vinblastine were detected by following the transitions 825.4 amu 765.4 and 811.3 to 751.4, respectively, using an API5000 triple quadrupole mass spectrometer (Applied Bioscience, Foster City, CA) in positive turbospray mode. Analyst software (AB Sciex, Foster City, CA) was used to reduce chromatography data.

For plasma samples, vincristine standard curves in plasma were used to assay both forms of vincristine sulfate studied with a linear range of detection from 2.5–10,000 ng/ml. For CSF samples the lower limit of detection for vincristine in the NHP CSF was 1 ng/ml. Quality controls met accuracy requirements defined by the 2001 FDA Guidance for Industry for Bioanalytical Method Validation.

Pharmacokinetic analysis

The plasma and CSF pharmacokinetic parameters for total (liposomal encapsulated, non-encapsulated, and protein bound) vincristine were analyzed by non-compartmental methods. The peak vincristine concentration (C_{max}) and time to peak concentration (T_{max}) were determined from the time and concentration data for each animal. Area under the concentration x time curve (AUC) was calculated using the linear trapezoidal method and extrapolated to infinity ($AUC_{0-\infty}$). The terminal half-life was calculated by dividing 0.693 by the terminal rate constant. Clearance (CL) was calculated by dividing the vincristine dose by the $AUC_{0-\infty}$.

Results

Vincristine and VSLI pharmacokinetic parameters are summarized in Table 1. Following a 15 min intravenous bolus of standard vincristine, a median maximum drug concentration (C_{max}) of 1070 ng/mL (range, 990–2100 ng/mL) was detected at the EOI. A multi-exponential plasma disappearance curve revealed a median initial (EOI to 30 min post-infusion) half-life ($t_{1/2}$) of 4.8 min (range, 4.4–5.0 min) and terminal $t_{1/2}$ of 24.3 h (range, 14.2–54.4 h). Median plasma exposure to vincristine as estimated by the $AUC_{0-\infty}$ was 614 ng•hr./mL (range, 336–676 ng•hr./ mL) and CL was 33.8 mL/min (range, 29.6–54 mL/min).

Following a 15 min bolus equivalent dose of VSLI, C_{max} was 4800 ng/ml (range, 3700–7300 ng/mL) at 15 min post EOI. In contrast to standard vincristine, which had a multi-exponential plasma disappearance curve, a near mono-exponential curve with a median $t_{1/2}$ of 17.9 h (range, 13.9–21.5 h) was calculated with VSLI. (Fig. 2a) Estimated plasma exposure to total vincristine was 195,000 ng•hr./ml (range, 163,000–312,000 ng•hr./mL) and CL was 0.098 mL/min (range, 0.073–0.113 mL/min). The ratios of the clearance of standard aqueous vincristine to that of VSLI ($CL_{VCR}:CL_{VSLI}$) for individual animals were 300, 463 and 477. Vincristine was not detected in any CSF sample after administration of either standard vincristine or VSLI.

Discussion

With the recent FDA approval of Marqibo ®/VSLI and active ongoing studies incorporating the use of VSLI for both relapsed and initial therapy for treatment of cancer, VSLI is being used more frequently as substitution for standard vincristine [13]. Hence, understanding the differences in pharmacokinetics between standard vincristine and VSLI will become increasingly important, especially in developing dosing schedules. Since the sphingomyelin/cholesterol liposome encapsulation can prolong the half-life, it will be important to consider how to optimally substitute VSLI in place of standard vincristine, considering the unique properties of VLSI [2–4]. Utilizing a non-human primate model, we were able to improve upon the understanding of VSLI pharmacokinetics in comparison to standard vincristine by facilitating a head-to-head direct comparison of both agents.

In three animals, each serving as their own control, we demonstrate that standard vincristine clearance exceeds that of VSLI by approximately 400-fold. This finding is consistent with results from our pediatric phase I study of VSLI where we demonstrated that the clearance of total vincristine was approximately 100-fold lower than historical standard vincristine values, [14, 15] and similar to findings in adult VSLI pharmacokinetic results [8]. Notably, this is first report looking at the CSF penetration of VSLI. As expected, based on prior studies of vincristine where CSF penetration has not been seen, [16] CSF was not observed for either standard vincristine or VSLI in the non-human primate model. This is both an especially salient feature given the known neurotoxicity associated with vincristine where CSF penetration would not be desired, but also noteworthy in that there would likely be a limited role in CNS directed therapy using either vincristine or VSLI.

There are a number of potential limitations of this study. Each NHP animal was dosed only once with VSLI, and given the slow clearance of VSLI, there remains the possibility that more frequent administration could lead to drug accumulation and cumulative toxicity, and that less frequent dosing for VSLI is needed than for standard vincristine. The liposomal formulation of VSLI was designed to prolong the circulation time of encapsulated vincristine, allowing for the gradual release of vincristine and enhancing the exposure time to this active chemotherapeutic agent [7, 8]. The pharmacokinetic assay employed in this study measured total vincristine and thus provided no information about the proportion of encapsulated versus free vincristine levels. Consequently, the total amount of active vincristine exposure from VSLI at any given time is not known, and the clearance of such free, active vincristine is likely to be unchanged compared to the standard aqueous formulation of vincristine. The ability to quantify both encapsulated and non-encapsulated forms of vincristine is currently under development, [17] and incorporating such data in future studies will further enhance the ability to understand VSLI pharmacokinetics and maximally incorporate this agent into treatment regimens. Finally, tissue penetration was not studied, which may be an especially notable feature of VSLI where deposition in target tissues could lead to prolonged release of the active component (i.e., vincristine) [7].

Preclinical and clinical activity of VSLI in adults with refractory cancers provides the rationale for further investigation of VSLI in clinical trials and pharmacokinetic data will best inform how to incorporate VSLI into combination chemotherapy regimens. Based on

our NHP model, providing in vivo pharmacokinetic analysis, we were able to demonstrate that there is no CSF penetration of VSLI and that clearance of VSLI is delayed compared to standard vincristine by approximately 400-fold. This information should be taken into account when planning for studies utilizing VSLI—and consider the possibility of drug accumulation and cumulative toxicity and whether the standard weekly dosing used for vincristine should be maintained for VSLI administration. Results from a recently completed phase III study to evaluate VSLI in the treatment of elderly subjects (≥ 60 years of age) with newly diagnosed ALL ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01439347) NCT01439347) which incorporates a head to head comparison of vincristine to VSLI are pending, but will be particularly informative to understand differences between vincristine and VSLI. Similar combination studies in adults are planned or ongoing. The pediatric development plan has included a phase I trial of VSLI for children with recurrent or refractory solid tumors, which was recently completed ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01222780) NCT01222780). Next steps include study of VSLI, as substitution for standard vincristine, with combination chemotherapy in children with relapsed/ refractory ALL. When available, future studies should incorporate quantifying both encapsulated and non-encapsulated forms of vincristine to enhance the ability to understand VSLI pharmacokinetics, with a particular focus on tissue penetration to evaluate for the possibility of prolonged release of active drug.

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Fig. 1.
Illustration of indwelling Pudenz 4th ventricular catheter attached to a subcutaneous Ommaya reservoir in a non-human primate

Fig. 2. Plasma concentration of vincristine following a 15 min intravenous infusion of 0.1 mg/kg dose VCR (open symbol) or VSLI (closed symbol) in non-human primates. VSLI: Vincristine elimination following a near mono-exponential disappearance curve. VCR: vincristine elimination demonstrates a multi-exponential plasma concentration-time curve. Initial $T_{1/2} = 3.0$ min (2.5–3.5 min). The ratios $Cl_{VCR}:Cl_{VSLI}$ for the individual NHP were 300, 463 and 477

Table 1

Median (range) pharmacokinetic parameters of standard vincristine and VSLI in non-human primates

Non-Human Primates						
<u>Vincristine preparation</u>	<u>No.</u>	<u>C_{max} (ng/mL)</u>	<u>AUC_(0-∞) (ng/mL•hr)</u>	<u>T_{max} (hr)</u>	<u>T_{1/2} (hr)</u>	<u>CL (mL/min)</u>
VCR (1.2 mg/m ²)	3	1070 (990–2100)	614 (336–676)	EOI	24.3 (14.2–54.4)	33.6 (29.6–54)
VSLI (1.2 mg/m ²)	3	4800 (3700–7300)	195,000 (163,000–312,000)	0.5 (0.33–0.5)	17.9 (13.9–21.5)	0.089 (0.073–0.113)

AUC(0–∞); area under the curve extrapolated to infinity; CL; clearance; C_{max}: maximum (peak) concentration; EOI: end of infusion; T_{1/2}: elimination half-life; T_{max}: time when the maximum plasma drug concentration is reached; VCR: standard aqueous vincristine formulation; VSLI: Vincristine Sulfate Liposomes Injection