Pharmacological Research 61 (2010) 385–390

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Diet-induced obesity alters vincristine pharmacokinetics in blood and tissues of mice

James W. Behan[a], Vassilios I. Avramis[b,c,d,e], Jason P. Yun[a], Stan G. Louie[f], Steven D. Mittelman[a,c,d,e,*]

[a] Division of Endocrinology, Diabetes & Metabolism, Childrens Hospital Los Angeles, 4650 Sunset Boulevard, Los Angeles, CA 90027, USA
[b] Division of Hematology & Oncology, Childrens Hospital Los Angeles, 4650 Sunset Boulevard, Los Angeles, CA 90027, USA
[c] Saban Research Institute, Childrens Hospital Los Angeles, 4650 Sunset Boulevard, Los Angeles, CA 90027, USA
[d] Department of Pediatrics, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA
[e] Norris Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA
[f] School of Pharmacy, University of Southern California, 1985 Zonal Avenue, Los Angeles, CA 90033, USA

ARTICLE INFO

Article history:
Received 21 December 2009
Received in revised form 11 January 2010
Accepted 12 January 2010

Keywords:
Obesity
Leukemia
Chemotherapy
Vincristine
Pharmacokinetics

ABSTRACT

Obesity is associated with poorer outcome from many cancers, including leukemia. One possible contributor to this could be suboptimal chemotherapy dosing in obese patients. We have previously found that vincristine (VCR) is less effective in obese compared to non-obese mice with leukemia, despite weight-based dosing. In the present study, we administered ^{3}H-VCR to obese and control mice to determine whether obesity would cause suboptimal VCR exposure. Blood VCR concentrations were fitted with a three-compartment model using pharmacokinetic analysis (two-stage PK) in three subsets of VCR concentrations vs. time method. Tissue and blood VCR concentrations were also analyzed using non-compartmental modeling. Blood VCR concentrations showed a triexponential decay and tended to be slightly higher in the obese mice at all time-points. However, the $t_{1/2,\beta}$ and $t_{1/2,\gamma}$ were shorter in the obese mice (9.7 min vs. 44.5 min and 60.3 h vs. 85.6 h, respectively), resulting in a lower $AUC_{0-\infty}$ (13,099 ng/mL h vs. 15,384 ng/mL h). Had the dose of VCR been "capped", as is done in clinical practice, the $AUC_{0-\infty}$ would have been 36% lower in the obese mice than the controls. Tissue disposition of VCR revealed a biexponential decay from spleen, liver, and adipose. Interestingly, VCR slowly accumulated in the bone marrow of control mice, but had a slow decay from the marrow in the obese mice. Thus, obesity alters VCR PK, causing a lower overall exposure in circulation and bone marrow. Given the high prevalence of obesity, additional PK studies should be performed in obese subjects to optimize chemotherapy dosing regimens.

1. Introduction

There is growing evidence that obesity is associated with increased mortality from cancer, including leukemia [1–4]. Increased leukemia mortality may be attributed in part to an increase in incidence in obese individuals [5,6]. However, patients who are obese at the time they are diagnosed with acute lymphoblastic leukemia or acute myeloid leukemia have significantly higher mortality than their normal weight counterparts [7–9]. There are a myriad of possible explanations for this, including delayed diagnosis, more aggressive cancer, poor compliance, and impaired immunity. It is also conceivable that dosing practices of chemotherapies, such as *a priori* dose reduction and dose capping may contribute to suboptimal levels in obese patients [10–12]. Obesity is a complex state associated with not only an increase in total body size, but also increased fat distribution, relative decrease in lean body mass [13], and altered cardiac [14], liver [15], and renal function [16,17]. Despite the importance of accurate chemotherapy dosing, and the increasing prevalence of obesity, few pharmacokinetic studies have been performed to elucidate the clinical impacts of obesity on chemotherapeutic agents pharmacokinetics and ultimate long term survival [18].

Vincristine (VCR), a potent antimicrotubule agent, is a seminal drug in the treatment of multiple cancers, including hematological malignancies, in children and adults [19,20]. The sensitivity of leukemia cells to VCR has been demonstrated to correlate with event-free survival [21,22]. We recently reported that obese mice transplanted with syngeneic leukemia cells exhibited impaired survival after VCR treatment when compared to control mice [23]. In

Abbreviations: AUC, area under-the-curve; HPLC, high-pressure liquid chromatography; LC/MS, liquid chromatograph/mass spectrometry; PK, pharmacokinetics; SA, specific activity; VCR, vincristine.

* Corresponding author at: Childrens Hospital Los Angeles, Division of Endocrinology, MS #61, 4650 Sunset Boulevard, Los Angeles, CA 90027, USA.
Tel.: +1 323 361 7653; fax: +1 323 906 8013.
E-mail address: smittelman@chla.usc.edu (S.D. Mittelman).

1043-6618/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phrs.2010.01.007

that study, we had dosed VCR in proportion to body weight, and thus effectively matched the blood and tissue VCR concentrations in the obese and control mice. However, clinical dosing of VCR in pediatric cancer patients is proportional to body surface area, and is generally "capped" at 1 m^2. Thus, an obese 18 year old generally receives approximately the same total VCR dose as an average 8–9 year old.

Recently, there have been many studies examining the pharmacokinetics of VCR in young children and adolescent ALL patients [24–27]. Some of these attempted to link the observed differences in the variable response (outcomes) post-combination regimens to VCR exposure. Based on detailed PK analyses, one study concluded that there is no pharmacokinetic rationale to "limit" the dose in adolescent ALL patients [25]. Although it is clear that variability in expression and polymorphisms of the CYP3A isoforms of the P450 metabolic complex system could account for some of the variability observed in VCR PK [28–30], it is likely that other factors, such as obesity and concomitantly administered medications, may also play a role in VCR exposure in patients.

Given that obese patients with leukemia and other cancers have poorer outcomes, and receive less VCR per kg body weight than lean patients, the present study was designed to elucidate whether diet-induced obesity alters the blood and tissue pharmacokinetics of VCR in mice.

2. Methods

2.1. Mouse model

C57Bl/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). Male mice were weaned at 4 weeks of age and fed a high fat diet (60% of calories from fat, Research Diets D12492, New Brunswick, NJ) or a control diet (10% of calories from fat, D12450), and used for experiments at 17 weeks of age. All animal experiments were approved by the Childrens Hospital Los Angeles Institutional Animal Care and Use Committee, and performed in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals.

2.2. VCR pharmacokinetics experiment

Twenty obese and 21 control mice received tail-vein injections of tritiated vincristine ([^{3}H]VCR) proportional to body weight (specific activity 75 μCi/mg; dose 0.5 mg/kg, American Radiolabeled Chemicals, Inc., St. Louis, MO). The VCR was synthesized by tritium gas catalyzed exchange, and therefore the molecule was uniformly labeled in random positions. Blood samples were collected from a subset of animals (n = 8 per group) from the submandibular venous plexus at t = 5 min using GoldenRod lancets (Medipoint, Inc., Mineola, NY) into EDTA coated tubes. Mice were sacrificed in groups of 3–5 at t = 0.25, 0.5, 1, 3, 8, and 24 h post-injection.

2.3. VCR measurements

Whole blood and tissue specimens were rapidly removed, and then solubilized using Solvable (PerkinElmer, Waltham, MA). The brown fat depot was removed from the intrascapular space and identified by its tan color. Whole blood, spleen, and liver were decolorized using 30% H_2O_2. Samples were then read on a scintillation counter (TriCarb 2100TR, PerkinElmer) using ReadySafe (Beckman Coulter, Inc., Fullerton, CA). Recovery was determined by spiking tissues (blood, spleen, and liver) from an un-injected mouse with a known quantity of ^{3}H-vincristine and processing in parallel with experimental samples.

Additional plasma samples from the 24 h time-point were used to validate the tritium assay by comparing the concentration using an LC/MS assay [31]. The evaporated samples from cellular extracts or media were then reconstituted in 50 μL of running buffer consisting of 45% methanol and 55% of 0.1% formic acid (v/v). The entire solution was vortexed for 1 min and then centrifuged at 13,000 rpm at 4 °C for 10 min. Afterward, the clarified supernatant was transferred into an injection vial, where 25 μL of the sample was injected into an Agilent 1100 HPLC system linked onto an API 3000 (Applied Biosystems, Foster City, CA). The analytes were separated using a C18 ACE column with the following dimensions 50 mm × 3.0 mm. The analytes were eluted using mobile phase consisting of 45% methanol and 55% of 0.1% formic acid (v/v), where the flow rate was set at 350 cc/min. The amount of VCR was determined using API 3000, where the m/z to transition ion was monitored 413.6 → 392.4 and 406.5 → 272.0 for vincristine and vinblastine, respectively. Using this method, we have previously measured VCR with the lowest level of quantification of 0.02 nM. The standard curve for the assay had an r^2 = 0.998.

2.4. Pharmacokinetic (PK)–analytical modeling

Total blood VCR levels were analyzed by the two-stage PK populations method using NONLIN software [32]. The data were fit to a three-compartment open pharmacokinetic model. Specifically, the equation used for the VCR concentration–time fit in blood is shown in Eq. (1):

$$C(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} + C \cdot e^{-\gamma t} \quad (1)$$

Compartmental modeling was also used to accommodate tissue VCR concentrations. It was apparent that the tissues tested achieved immediate equilibrium, i.e. within 15 min. The only exception was the bone marrow. Hence, the equation describing the fate of VCR after the first 15 min in spleen, liver, fat, and brown fat is:

$$C(t)_{\text{tissue}} = B \cdot e^{-\beta t} + C \cdot e^{-\gamma t} \quad (2)$$

For bone marrow, the VCR concentration showed an initial accumulation phase in both groups, but a slower decay in the obese mice, and a slow accumulation in the lean mice. Thus, the equations describing bone marrow concentrations were:

$$C(t)_{\text{obese-marrow}} = B \cdot e^{+\beta t} + C \cdot e^{-\gamma t}, \text{ and} \quad (3a)$$

$$C(t)_{\text{nonobese-marrow}} = B \cdot e^{+\beta t} + C \cdot e^{+\gamma t}, \text{ for the lean mice.} \quad (3b)$$

Initial estimates from this compartmental model were then used to derive a non-compartmental analysis using customized subroutines based on the methods previously described [32,33]. Using these methods, blood VCR clearance was estimated by the equation:

$$\text{total body clearance}\,(\text{CL}_\text{T}) = \frac{\text{dose}}{\text{AUC}_{0-\infty}} \quad (4)$$

The apparent volume of the central compartment of distribution (V_{DC}) and apparent volume of distribution at steady-state (V_{DSS}) were, respectively, calculated by the following equations:

$$V_{\text{DC}} = \frac{\text{dose}}{A + B + C}, \text{ and} \quad (5)$$

$$V_{\text{DSS}} = \frac{\text{dose}[(A/\alpha^2) + (B/\beta^2) + (C/\gamma^2)]}{(\text{AUC}_{0-\infty})^2} \quad (6)$$

Finally, a limited physiological pharmacokinetic model was derived from the blood and tissue compartmental modeling parameters.

The averages per time-point VCR concentrations were analyzed by a NONMEM three-compartment open model subroutine. The solutions were super-imposable to the two-stage compartmental method described above, thus validating our solution. Moreover,