Published in final edited form as:

J Pharmacokinet Pharmacodyn. 2018 October ; 45(5): 733–746. doi:10.1007/s10928-018-9603-z.

Physiologically-based pharmacokinetic and pharmacodynamic models for gemcitabine and birinapant in pancreatic cancer xenografts

Xu Zhu,

Department of Pharmaceutical Sciences, University at Buffalo, The State University of New York

Sheryl Trueman,

Department of Pharmaceutical Sciences, University at Buffalo, The State University of New York

Robert M. Straubinger, and

Department of Pharmaceutical Sciences, University at Buffalo, The State University of New York

William J. Jusko*

Department of Pharmaceutical Sciences, University at Buffalo, The State University of New York

Abstract

The anticancer effects of combined gemcitabine and birinapant were demonstrated as synergistic in PANC-1 cells *in vitro*. In this study, pharmacokinetic information derived from experiments and the literature was utilized to develop full physiologically-based pharmacokinetic (PBPK) models that characterize individual drugs. The predicted intra-tumor drug concentrations were used as the driving force within a linked PBPK/PD model for treatment-mediated changes in tumor volume in a xenograft mouse model. The efficacy of the drug combination *in vivo* was evaluated mathematically as exhibiting additivity. The network model developed for drug effects in the *in vitro* cell cultures was applied successfully to link the *in vivo* tumor drug concentrations with tumor growth inhibition, incorporating more mechanistic features and accounting for disparate drug interaction outcomes *in vitro* and *in vivo*.

Keywords

Physiologically-based pharmacokinetic model; pharmacodynamics; gemcitabine; birinapant; drug combination; pancreatic cancer xenografts

Introduction

Pancreatic cancer is the fourth leading cause of cancer-related deaths in the United States and is characterized by low survival rates and high drug resistance [1]. Numerous efforts have sought to identify efficacious drug combinations for treatment of pancreatic cancer.

*Corresponding author, Dr. William J. Jusko, 404 Kapoor Hall, Buffalo, NY 14214-8033, wjjusko@buffalo.edu, Phone: (716) 645-2855, Fax: (716) 829-6569.

Conflict of Interest/Disclosure

The authors declared no conflict of interest.

Preclinical systems are important tools for assessment of novel drug combinations and understanding mechanisms of drug action and interactions.

Gemcitabine (2',2'-difluoro-2'-deoxycytidine (dFdC); Gemzar) is a cytidine analog that has clinical activity in a wide variety of solid tumors, including pancreatic, ovarian, breast, and non-small-cell lung cancers. The main dose-limiting toxicity of gemcitabine is myelosuppression [2]. The uptake of gemcitabine into cells is *via* nucleoside transporters (SLC28/29), and it is phosphorylated intracellularly by deoxycytidine kinase into its active diphosphate (dFdCDP) and triphosphate (dFdCTP) forms. The metabolites dFdCDP inhibits ribonucleotide reductases, whereas dFdCTP competes with the natural substrate dCTP for incorporation into DNA, resulting in inhibition of DNA synthesis [3, 4]. Gemcitabine is administered by intravenous infusion. The volume of distribution increases with infusion duration, from 50 L/m² to 370 L/m², and plasma protein binding is negligible [2]. Gemcitabine is extensively metabolized to 2',2'-difluoro-2'-deoxyuridine (dFdU) by cytidine deaminase (CDA), followed by urinary excretion [2]. The plasma half-life is about 30 min and this short half-life is the result of extensive CDA expression in liver and other tissues, as well as rapid tissue distribution [5, 6].

Birinapant (TL32711) is a bivalent second mitochondria-derived activator of caspase (SMAC) mimetic or Inhibitor of Apoptosis Proteins (IAP) antagonist. It is currently being tested clinically in combination with pembrolizumab for solid tumors and with carboplatin for ovarian, fallopian tube, and primary peritoneal cancers. Birinapant binds with high affinity to several IAPs, including XIAP and cIAP1,2, and rapidly degrades TRAF2-bound cIAP1,2, thereby inhibiting TNF-mediated NF- κ B activation. In addition, birinapant promotes the activation of caspases. Birinapant has shown synergistic cytotoxicity with several widely used chemotherapeutic agents in preclinical studies [7]. Compounds from the SMAC mimetic drug class exhibit similar pharmacokinetic features, which include dose-linear exposure, moderate plasma half-life, and extensive distribution in tissues [8–11]. In a phase I clinical trial, birinapant was administered by intravenous infusion and the plasma concentration profile showed a tri-exponential decline, with an estimated terminal half-life of 19–47 h. The volume of distribution was high (~710.4 L), indicating extensive tissue uptake, and the urinary excretion was low [12].

In previous cell culture studies, the efficacy of combined gemcitabine and birinapant was determined as synergistic in PANC-1 cells [13], and a systems network model was developed to characterize quantitatively the mechanisms of drug action upon several biological signaling pathways [14, 15]. In this study, the efficacy of this drug combination was evaluated in xenograft animal models, and mechanistic modeling of drug interaction pathways was further incorporated to elucidate the events between dosing and efficacy. Characterization of drug distribution at the site of action (e.g. tumor) is another important step in evaluating tumor growth inhibition and for translating between *in vitro* and *in vivo* research. Therefore, PBPK and network modeling approaches, based on experimental studies and information gathered from the literature, were utilized to characterize drug concentrations in plasma and tumor, and effects on tumor growth.

Materials and Methods

Data Sources

The data sources used to characterize the pharmacokinetics (PK) of gemcitabine (dFdC) and its metabolite dFdU are summarized in Table S-1 [5, 6, 16, 17]. Concentration *vs.* time profiles in plasma and tissues were available for doses of 0.1, 20, 30, 160 mg/kg in mice. Distribution partition coefficients (K_p) values were calculated from the ratio of the AUC in tissue to the AUC in plasma for tissues for which concentration profiles were not available [5].

Concentration *vs.* time profiles of birinapant in plasma, tumor (MDA-MB-231 xenografts), liver, kidney, lung, heart, brain, and intestine were obtained from a published poster [18] for a single intravenous (IV) bolus dose in mice (5 mg/kg). Birinapant concentrations in tumor and liver of mice bearing patient-derived pancreatic cancer xenograft tumors were also obtained from a published poster [19]. Drug concentrations in tissues were converted from ng/g to ng/ml by assuming a tissue density of 1 g/ml.

Study design for tumor growth in vivo

In vivo studies were performed by Tetralogic Pharmaceuticals Inc. PANC-1, a pancreatic cancer cell line originating from a 56 year-old Caucasian male, was cultured in Dulbecco's Modified Eagle's Medium (DMEM; Cellgro) supplemented with 10% (v/v) fetal bovine serum (FBS; Atlanta Biologicals). Female athymic *nu/nu* mice (Harlan Sprague-Dawley) weighing approximately 20–24 g were injected subcutaneously in the flank with 1×10^7 cells suspended in 200 μ l of a solution containing 1:1 Hank's buffered saline to Matrigel. When tumors reached approximately 150 mm³, animals were randomized by tumor volume into groups of 10 mice each, and dosing was initiated (Day 0). Tumor dimensions were measured at least twice weekly by digital caliper (Fowler Ultra-Cal IV, Newton, MA). Tumor volumes were calculated using the formula: $TV = width^2 \times length \times 0.52$.

Birinapant was administered by intraperitoneal (IP) injection at 6, 20, or 60 mg/kg in 12.5% Captisol in sterile water (pH~4) once every 3 days for 10 total treatments (q3d $\times$ 10). A vehicle control group received Captisol without drug using the same dosing schedule. Gemcitabine was administered IP at 40 mg/kg once every 3 days for 4 doses beginning on Day 0, and the cycle was repeated on day 15 (q3d $\times$ 4 $\times$ 2). The drug combination group received 20 mg/kg birinapant (q3d $\times$ 10) and 40 mg/kg gemcitabine (q3d $\times$ 4 $\times$ 2).

Physiologically-based pharmacokinetic (PBPK) models

The workflow of model development is depicted in Figure 1. Physiologic parameters were obtained from the literature (Table 1) [20–22]. Mass balance was maintained throughout the system. Both drugs are rapidly absorbed after IP injection, and in-house data showed that the bioavailability of birinapant by IP injection is close to 100% (data not shown). Plasma protein binding, which is negligible for gemcitabine [5], was not considered.

The PBPK model schematic for birinapant is presented in Figure 2. The model includes 9 compartments, blood (*pla*), lung (*lug*), tumor (*tum*), intestine (*int*), liver (*liv*), kidney (*kid*),

heart (*hrt*), brain (*bra*), and the carcass remainder (*res*), linked by blood flow (Q). The concentrations in plasma and lung were described as:

$$V_{pla} \frac{dC_{pla}}{dt} = Q_{tum} C_{tum} + Q_{liv} \frac{C_{liv}}{Kp_{liv}} + Q_{kid} C_{kid} + Q_{hrt} C_{hrt} + Q_{bra} C_{bra} + Q_{res} C_{res} - Q_{lug} C_{pla} \quad (1)$$

$$V_{lug} \frac{dC_{lug}}{dt} = Q_{lug} (C_{pla} - \frac{C_{lug}}{Kp_{lug}}) \quad (2)$$

Elimination of the drug was assumed to occur in the liver and was modeled as a linear process with a clearance of CL_{liv} , and the concentrations in liver was described as:

$$V_{liv} \frac{dC_{liv}}{dt} = (Q_{liv} - Q_{int}) \frac{C_{lug}}{Kp_{lug}} + Q_{int} \frac{C_{int}}{Kp_{int}} - (Q_{liv} + CL_{liv}) \frac{C_{liv}}{Kp_{liv}} \quad (3)$$

Drug distribution in intestine was assumed to occur instantaneously and homogenously, and therefore perfusion-limited models were used to describe tissue concentrations:

$$V_{int} \frac{dC_{int}}{dt} = Q_{int} (\frac{C_{lug}}{Kp_{lug}} - \frac{C_{int}}{Kp_{int}}) \quad (4)$$

In other organs (tumor, kidney, heart, brain, and carcass), diffusion-limited models were:

$$(F_{i,vs} V_i) \frac{dC_{i,vs}}{dt} = Q_i \left(\frac{C_{lug}}{Kp_{lug}} - C_{i,vs} \right) - PS_i (C_{i,vs} - \frac{C_{i,evs}}{Kp_i}) \quad (5)$$

$$(1 - F_{i,vs}) V_i \frac{dC_{i,evs}}{dt} = PS_i (C_{i,vs} - \frac{C_{i,evs}}{Kp_i}) \quad (6)$$

$$C_i = F_{i,vs} C_{i,vs} + (1 - F_{i,vs}) C_{i,evs} \quad (7)$$

where Q_{tissue} is the organ blood flow rate, V_{tissue} is organ volume, $C_{tissue,vs}$ is the concentration in tissue vascular space, $C_{tissue,evs}$ is the concentration in tissue extravascular space, $F_{tissue,vs}$ is the fraction of vascular space volume, Kp_{tissue} is the partition coefficient, and PS_{tissue} is the permeability surface area product.

The PBPK model schematic for gemcitabine is presented in Figure 3. The model includes 11 compartments for the parent drug (dFdC): blood, lung, heart, tumor, intestine, pancreas (*pan*), spleen (*spl*), liver, kidney, bone marrow (*bom*), and the carcass remainder.

The concentrations in plasma and lung were described in the same format as in Eq. 1 and 2. Well-stirred perfusion-limited models were applied to tumor, intestine, pancreas, heart, and bone marrow because of the similar T_{max} and terminal half-lives in these organs compared to plasma. Diffusion-limited conditions were assumed for spleen and carcass.

The concentrations of dFdC in the liver and kidney were described as:

$$V_{liv} \frac{dC_{liv}}{dt} = (Q_{liv} - Q_{int} - Q_{pan} - Q_{spl}) \frac{C_{lug}}{Kp_{lug}} + Q_{int} \frac{C_{int}}{Kp_{int}} + Q_{pan} \frac{C_{pan}}{Kp_{pan}} + Q_{spl} C_{spl,vs} - (Q_{liv} + CL_{liv}) \frac{C_{liv}}{Kp_{liv}} \quad (8)$$

$$V_{kid} \frac{dC_{kid}}{dt} = Q_{kid} \left(\frac{C_{lug}}{Kp_{lug}} - \frac{C_{kid}}{Kp_{kid}} \right) - CL_{kid} \frac{C_{kid}}{Kp_{kid}} \quad (9)$$

The model also includes 5 compartments for the major metabolite dFdU: blood, lung, liver, kidney and the carcass remainder. The parent drug dFdC is metabolized to dFdU mainly in liver and kidney, and dFdU is subsequently excreted by the kidney.

The concentrations of dFdU in liver and kidney were described as:

$$V_{liv} \frac{dC_{U,liv}}{dt} = CL_{liv} \frac{C_{liv}}{Kp_{liv}} + Q_{liv} \left(\frac{C_{U,lug}}{Kp_{U,lug}} - \frac{C_{U,liv}}{Kp_{U,liv}} \right) \quad (10)$$

$$V_{kid} \frac{dC_{U,kid}}{dt} = CL_{kid} \frac{C_{kid}}{Kp_{kid}} + Q_{kid} \left(\frac{C_{U,lug}}{Kp_{U,lug}} - \frac{C_{U,kid}}{Kp_{U,kid}} \right) - CL_{U,kid} \frac{C_{U,kid}}{Kp_{U,kid}} \quad (11)$$

Pharmacodynamic (PD) model

The unperturbed growth of tumor was described with an exponential function:

$$\frac{dTV}{dt} = k_{ng} \cdot TV; \quad TV(0) = TV_0 \quad (12)$$

where k_{ng} is the first-order rate constant of net tumor growth, and TV_0 is the tumor volume when dosing was started.

The inhibitory effect of birinapant on tumor growth was described with a nonlinear function:

$$\frac{dTV}{dt} = k_{ng} \cdot TV - \frac{Kmax_b \cdot C_{b,tumor}}{KC50_b + C_{b,tumor}} \cdot TV; \quad TV(0) = TV_0 \quad (13)$$

and that of gemcitabine with a linear function:

$$\frac{dTV}{dt} = k_{ng} \cdot TV - Kill_g \cdot C_{g,tumor} \cdot TV; \quad TV(0) = TV_0 \quad (14)$$

where $Kmax_b$ is the maximal cell killing effect from birinapant, $KC50_b$ is the birinapant concentration in tumor required for half maximum effect; and $Kill_g$ is the first-order rate constant for killing caused by gemcitabine.

In the combination group, the interaction term Ψ was multiplied with either $KC50_b$ (Eq. 15) or $Kill_g$ (Eq. 16) to test the possibility of synergism, additivity, or antagonism of the two drugs.

$$\frac{dTV}{dt} = k_{ng} \cdot TV - \left(\frac{Kmax_b \cdot C_{b,tumor}}{KC50_b \cdot \Psi + C_{b,tumor}} + Kill_b \cdot C_{g,tumor} \right) \cdot TV \quad (15)$$

$$\frac{dTV}{dt} = k_{ng} \cdot TV - \left(\frac{Kmax_b \cdot C_{b,tumor}}{KC50_b + C_{b,tumor}} + (Kill_g \cdot \Psi) \cdot C_{g,tumor} \right) \cdot TV \quad (16)$$

When Ψ was multiplied with $KC50_b$, $\Psi < 1$ represents synergism, $\Psi = 1$ additivity, and $\Psi > 1$ antagonism; when Ψ was multiplied with $Kill_g$, the interpretations of Ψ are the opposite.

Implementation of network model to link PK and PD

A previously-developed network model describing drug-mediated changes observed *in vitro* at the proteomic level [13, 14] was used to link the drug concentrations in tumor and tumor growth to incorporate mechanistic features. Several parameters were modified to reconcile the differences between *in vitro* and *in vivo* systems (model schematic in Figure S-1 and model code in Supplementary Material). The parameters R_0 (the initial tumor size), and $IR50$ (the growth restriction on proliferating cells), were adjusted to account for the differences of tumor cell growth rate *in vivo* vs. *in vitro*. A fractional parameter F_{kg} was multiplied with the rate constants k_1 (transition from G0/G1 to S cell-cycle phases), k_2 (transition from S to G2/M phases), k_3 (transition from G2/M to G0/G1 phases), k_{apo} (rate from proliferating to apoptotic death), and k_{other} (rate from proliferating to non-apoptotic death). Factor $(Dose/Fdose)$ was applied to modify the apoptosis signaling induced by birinapant to account for dose-dependent tumor inhibitory effects. These parameters were calibrated so that the tumor growth profiles were captured well for the different doses of

birinapant and gemcitabine, alone and in combination. The drug concentration units were also converted from those in the xenograft study (ng/ml) to those in the cell culture study (nM).

Prediction of K_p values

The Rodgers et al method for moderate and strong bases was used to calculate K_p values of birinapant distribution in different tissues from *in silico* data [23]. The pK_a of birinapant was estimated using JChem (<https://epoch.uky.edu/ace/public/pKa.jsp>), and the $\log KOWWIN$ value was predicted using EPIWEB 4.1 (US Environmental Protection Agency, Washington DC). Compounds from the same drug class as birinapant, such as AT-406 and GDC-0512, share similar properties of plasma protein binding and blood/plasma partitioning, and therefore the f_{up} and $K_{b/p}$ values of birinapant were assumed to be similar to AT-406 [24, 25]. All other system-related distribution parameters were obtained from the literature [23].

Data analysis

Model fitting and parameter estimation was performed using ADAPT 5 with the maximum likelihood algorithm [26]. Naïve-pooled data from all replicate studies were analyzed using the variance model:

$$V_i = (\text{intercept} + \text{slope} \cdot Y(t_i))^2 \quad (18)$$

where V_i is the variance of the response at the i th time point (t_i), and $Y(t_i)$ is the predicted response at time t_i . The variance parameters *intercept* and *slope* were estimated together with system parameters. Model performance was evaluated by goodness-of-fit criteria, including visual inspection of the fitted curves, sum of squared residuals, Akaike Information Criteria (AIC), Schwarz Criterion (SC), and Coefficients of Variation (CV%) of the estimated parameters.

Results

PBPK model for birinapant

The concentration profiles of birinapant in plasma, tumor (MDA-MB-231 xenografts), and six other tissues after IV injection of 5 mg/kg are presented in Figure 4. Linear pharmacokinetics were assumed based on information provided in a published poster [27]. Birinapant was assumed to be eliminated mainly by the liver, similar to drugs of the same class [25]. Based on this information, a PBPK model for birinapant was developed (Figure 2). The terminal half-life was about 20 h in plasma and 40–200 h in tissues, indicating slow drug loss from tissues. Therefore, diffusion/permeability-limited distribution models were applied to the major organs, and favorable PK features resulting in appreciable concentrations in tumor could be presumed. The concentration profiles were captured well by the PBPK model (Figure 4), and the parameters, estimated with reasonable CV%, are summarized in Table 2. Additional model goodness-of-fit plots are provided in Supplementary File 2. The estimated hepatic intrinsic clearance was 105 ml/h, and accounting for the hepatic blood flow of 135 ml/h, the apparent clearance calculated from

$\frac{Q_H \cdot CL_{int}}{Q_H + CL_{int}}$ is about 60 ml/h. If converted to a 75 kg human using allometric scaling with a coefficient factor of 0.75, the estimated clearance would be about 24 L/h, which matches the reported value from human studies [28]. The estimated partition coefficients values (K_p) for most tissues are large (10–200), except for brain (<2), consistent with the characteristic of extensive tissue distribution for this drug class. Desmoplastic stroma and inadequate vasculature in pancreatic cancer lead to relatively lower tumor distribution in pancreatic cancer xenografts compared to breast cancer xenografts [29], and a correlation of liver and tumor concentrations (Figure S-2) indicated concentrations about 20-fold lower in pancreatic cancers than in liver [19]. Therefore, the K_p value for tumor tissue in the pancreatic cancer xenografts was calibrated to 2.0, so that tumor concentrations 20-fold lower than in liver were predicted (Figure 4).

The model-estimated tissue K_p values were compared with the K_p values predicted with the Rodgers et al method for strong bases [23]. Physicochemical parameters of birinapant required for K_p prediction were calculated: the pKa using JChem was 12.6, and the $\log KOWWIN$ was 2.43. Based on these values, the Rodgers et al method [23] predicted K_p values that are about 10-fold lower than the model-estimated values for tissues other than the heart and brain (Table 2).

PBPK model for gemcitabine

Over a range of doses (0.1–160 mg/kg), the plasma concentrations of the parent drug gemcitabine (dFdC) indicated linear PK (Figure 5). More than 90% of gemcitabine was recovered in urine, mainly as the dFdU metabolite, and protein binding of gemcitabine is negligible [5]. Concentration profiles of dFdC in pancreatic xenograft tumors, bone marrow, and spleen were obtained from literature (Figure 5). For the other tissues for which full concentration profiles were not available, such as lung, heart, intestine, pancreas, liver, and kidney, the reported tissue T_{max} and terminal half-lives were similar to those of plasma [5], and therefore well-stirred and perfusion-limited distribution was assumed for these tissues. Because of the extensive expression of CDA (the major metabolizing enzyme for dFdC) in liver and kidney of mice, and the high K_m value (the dFdC concentration required for half-maximal saturation of the CDA enzyme) [30], linear elimination of dFdC and conversion to dFdU in liver and kidney were assumed. A PBPK model characterizing both dFdC and dFdU was developed based on this information (Figure 3). Concentrations of dFdC and dFdU were well characterized by the model (Figure 5) with reasonable parameter estimates (Table 3). The apparent hepatic clearance is estimated as 12.4 ml/h and renal clearance as 2.9 ml/h using the previous equation, and the estimated total clearance is 15.3 ml/h. The higher clearance in liver compared to kidney is consistent with greater expression of CDA in liver [31]. The K_p values were 0.137 in tumor and 0.498 in bone marrow, indicating limited drug distribution into the site of action and slightly higher distribution into the major site of toxicity.

Tumor growth analysis

Unperturbed tumor growth was best fitted with an exponential function. The inhibitory effect of birinapant on tumor growth was described with a nonlinear function because of

available information for a range of dosages, whereas the inhibitory effect of gemcitabine was described with a linear function. To achieve more accurate estimation of the drug interaction term Ψ , the control group and the single-agent treatment groups were modeled simultaneously (Eq. 12–14). Next, the parameters for tumor growth and single drug efficacy were fixed, and the interaction term Ψ was estimated to characterize tumor growth for the drug combination (Eq. 15,16). The tumor growth profiles were well described with this sequential model-fitting strategy (Figure 6) and the parameters were estimated with reasonable values and CV% (Table 4).

The PANC-1 cell line was used for both the *in vivo* tumor growth study and our previous cell culture studies [13, 14]. Therefore, the parameters obtained from the two systems were compared. The estimated rate of net tumor growth *in vivo* (k_{ng}) was 0.00571 h^{-1} , corresponding to a doubling time of 5 days. This rate is much smaller than the net cell growth rate *in vitro* ($k_g - k_d$), which was about 0.02 h^{-1} [13]. Comparing the *in vivo* and *in vitro* efficacy of birinapant, the maximal rate of cell killing ($Kmax_b$) *in vivo*, was 0.0167 h^{-1} ; whereas $Kmax_b$ *in vitro*, calculated from ($Imax \cdot k_d$) or ($Smax \cdot k_d$), was around 0.007 h^{-1} . The $KC50_b$ was $2.90 \times 10^3 \text{ ng/ml}$ ($3.60 \times 10^3 \text{ nM}$) *in vivo*, whereas $IC50$ or $SC50$ was around 150 nM *in vitro*. These indicate that PANC-1 cells are about 20-fold more sensitive to birinapant *in vitro* than *in vivo*. The rate of cell killing induced by gemcitabine ($Kill_g$) was $2.76 \times 10^{-5} \text{ h}^{-1}$ *in vivo*, and the corresponding *in vitro* parameter, calculated from ($Imax \cdot k_g / IC50$), was 0.001 h^{-1} . Therefore, PANC-1 cells are 30-fold more sensitive to gemcitabine *in vitro* than *in vivo*. The interaction term Ψ *in vivo* was estimated with good accuracy: when Ψ was multiplied with $KC50_b$, the estimated Ψ was 1.27; when Ψ was multiplied with $Kill_g$, the estimated Ψ was 0.944 (Table 4). Neither Ψ value was significantly different from 1, which indicates additive effects of the drugs on tumor growth inhibition *in vivo*. In contrast, the estimated Ψ value *in vitro* was about 0.6, indicating synergism [13].

Bridging PK and tumor growth with a mechanistic network model

The mechanisms of action and interaction of gemcitabine and birinapant have been characterized previously using a systems network model based on cell culture data [14, 15]. Signaling pathways of cell cycle progression, DNA Damage Responses, MAPK, NF- κ B, and apoptosis were described quantitatively to drive changes in cell cycle progression and apoptosis, and were linked to changes in cell number. Because the same cell line was used as a xenograft model, the previously-developed network model [14] was applied to link tumor drug concentrations to tumor growth. More specifically, tumor concentrations were used to drive changes in network signaling that, in turn, led to changes in tumor volume. Several parameters were calibrated to account for the different growth rates and sensitivities to drug *in vitro* and *in vivo*, so that the tumor growth profiles for the vehicle control and different doses of gemcitabine and birinapant, alone and in combination, were all reasonably captured (Figure 7a left). The initial tumor size R_0 was calibrated to 150 mm^3 , the fractional parameter on growth and apoptosis rate F_{kg} was set to 0.15, and $IR50$ was 1500 mm^3 , so that the control tumor growth was captured well. The parameter for birinapant dose-dependent effect in apoptosis induction F_{dose} was calibrated to 50 mg/kg , so that the tumor growth profiles at different doses of birinapant were also well-captured. The combined effects of birinapant at 20 mg/kg and gemcitabine at 40 mg/kg were then simulated. The predicted

tumor growth profile matched the experimental data well, supporting the use of the network model, with these calibrated parameters, for linking PK and PD *in vivo*.

The network model can incorporate mechanistic features and provide additional insights into drug combination effects. For example, tumor growth profiles were simulated for both simultaneous and sequential dosing, motivated by *in vitro* studies that showed enhanced efficacy when birinapant exposure was delayed 24 h after initiating gemcitabine treatment [13]. Model simulations predicted that simultaneous dosing would be slightly more effective than sequential dosing in terms of overall tumor progression *in vivo* (Figure 7a *right*), in contrast to the *in vitro* results. Several major response signaling pathways were also simulated for the two dosing schedules. Similar apoptotic signaling was achieved with both dosing schedules (Figure 7b). However, the DNA repair response, which is induced by gemcitabine and contributes to cell survival, was stronger with sequential dosing (Figure 7c), and this may account for the different model-predicted efficacy *in vivo* compared to *in vitro*. Delayed exposure to birinapant was hypothesized to prolong the delay of DNA repair *in vitro* [13]. However, gemcitabine concentrations in tumor (100–1000 nM) were much higher than the concentrations *in vitro* (< 20 nM), and after an initial delay in onset, DNA repair signaling would be constantly activated *in vivo*. Therefore, although delayed birinapant dosing may initially mediate stronger suppression of DNA repair signaling, at later time points the effect may not be as strong as with simultaneous dosing (Figure 7c).

Discussion

In this study, pharmacokinetic information was gathered from the literature and experimental sources, and full-PBPK models were developed to characterize the PK features of birinapant and gemcitabine. Gemcitabine has been the first-line treatment for pancreatic cancer for years, but its PK has not been thoroughly described quantitatively. Here, gemcitabine distribution to sites of both action (tumor) and toxicity (bone marrow) was well characterized, and potentially could be linked to evaluation of toxicity. The relationship between plasma and tumor concentrations was derived for both drugs. Plasma and pancreatic tumor concentrations of birinapant were comparable, whereas gemcitabine concentrations were about 10-fold lower in tumors compared to plasma. In clinical trials, the plasma concentrations of birinapant attained were 100–1000 ng/ml [28], whereas gemcitabine plasma concentrations were found to exceed 10 µg/ml [32–34]. Therefore, the selected doses and the developed PK models have clinical relevance for drug exposures.

The estimated K_p values of birinapant in tissues other than brain or pancreatic cancer xenografts were 10–200, indicating extensive tissue accumulation of birinapant. However, the K_p values predicted using the Rodgers et al method [23] were all smaller than 15. Although this calculation method considers drug ionization and binding to different lipids and phospholipids, the predictions for birinapant were low. The large discrepancy is arguably the result of either specific binding of birinapant to its target IAP proteins or lysosomal trapping, which affects strongly lipophilic basic drugs significantly [35].

The data characterization and estimated parameters of the PBPK model were also compared to compartmental models (Figure S-3, S-4). The estimated central (V_I) and peripheral

volumes of distribution (V_d) for birinapant are much larger than those for gemcitabine, consistent with the large K_p values for birinapant. With compartmental modeling, the terminal plasma concentrations for birinapant were characterized better and the estimated clearance is higher (90 ml/h). However, the estimated clearance using the PBPK model is more consistent with that observed in humans. Theoretically the terminal phase in plasma and other tissues should be parallel. However, the observed late phase in plasma is faster than in other tissues. It is possible that the true terminal phase in plasma was not captured because of the lower limit of quantification or insufficient sampling. Therefore, with PBPK modeling, distribution between plasma and other tissues may provide better characterization of true terminal elimination. For gemcitabine, the terminal plasma phase was also captured better with compartmental modeling, and the estimated terminal elimination rate (1.4 h^{-1}) is much faster than that based on PBPK modeling (0.61 h^{-1}). The same reasoning as for birinapant may apply. The evidence in support is that if the last concentration value was removed and data re-fitted with compartmental modeling, the estimated slope (0.96 h^{-1}) is closer to that obtained by PBPK modeling (Figure S-5). It is also possible that current assumptions regarding gemcitabine elimination are inadequate. It was assumed that CDA expression is mainly in liver and kidney, and gemcitabine is eliminated by conversion to dFdU with a linear conversion rate. However, other elimination pathways may exist.

Comparing the estimated parameters obtained from *in vitro* and *in vivo* PD models that employ the same cancer cells, the cells grow faster and are more drug-sensitive *in vitro*. These factors must be considered when pursuing *in vitro*-to-*in vivo* translational research. Based on the semi-mechanistic PBPK/PD model, the estimated drug interaction term Ψ , whether multiplied with $KC50_b$ or $Kill_g$, was not significantly different from 1, indicating additive effects of the two drugs *in vivo*. However, this drug combination was synergistic *in vitro* using the same cell line [13]. Reasons for this difference were explored by incorporating additional mechanistic features. The network model was used to link the tumor concentrations and tumor growth, but several parameters required calibration to capture the tumor growth profiles for different birinapant doses, the single gemcitabine dose, and the combination. A dose-dependent factor, $(Dose/Fdose)$, was incorporated into the birinapant-induced apoptosis signaling in order to account for the dose-dependent tumor inhibitory effects, and $Fdose$ was calibrated to be 50 mg/kg. Therefore, this correction factor was less than 1 at the dose of 20 mg/kg used in the combination, representing weaker apoptosis signaling at this *in vivo* dose compared to cell culture studies. This model behavior may partially account for the observed disparity in overall efficacy of the drug combination *in vivo* vs. *in vitro*, and a larger range of doses for the combination *in vivo* could provide better characterization of drug interactions. A similar disparity in *in vitro* vs. *in vivo* drug efficacy has been reported for preclinical studies of the histone deacetylase inhibitor belinostat in pancreatic cancer [36]. Other factors that may also impact *in vitro*-to-*in vivo* translation of drug efficacy include the heterogeneity of cells in tumor tissue [37], as well as the influence of the stromal cells and extracellular matrix [38].

The protein binding of birinapant was not included in the model for lack of data. However, the distribution coefficient K_p was estimated, and therefore differences between tissue and plasma protein binding were partially accounted for. In addition, PK interactions of the drugs were not explored in the *in vivo* study. A clinical study found that gemcitabine

increased the efflux rate of birinapant from fast-distribution tissues to plasma (parameter k_{31} in a compartmental model) by 30%, possibly because of reduced OATP1B3-mediated tissue uptake [28]. The impact of gemcitabine on birinapant PK was considered as minor, and PK interactions were not considered here, because the final PK model structures for combined birinapant and gemcitabine were the same as for the single agents [28], and birinapant exhibited high concentrations in most tissues. Finally, the strategy here to translate the network model from *in vitro* to *in vivo* is empirical and specific to these two drugs. However, the overall approach may serve as a prototype for the evaluation of other drug combinations.

A Monte Carlo simulation of the drug effects was applied to address possible variation of parameters in altering the PD outputs. In our previous application of the network model to the effects of gemcitabine, a sensitivity analysis was carried out. We evaluated the network model with perturbation of key model parameters by both drugs and the outcome remained quite similar as reported [15]. Key PD outputs simulated with variability in the most sensitive parameters were presented in Figure S-6.

In conclusion, this study gathered literature and experimental information to develop full-PBPK models that characterize drug PK in sites of both action and toxicity. Drug efficacy in tumor growth inhibition *in vivo* was evaluated based on a semi-mechanistic PBPK/PD model. By bridging PK and PD with network signaling, we can interpret the observed results and evaluate drug combinations for other dosing schedules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by National Institutes of Health grants R01 GM24211 to WJJ and R01 CA198096 to RMS. We thank Tetralogic Pharmaceuticals Inc. for sharing the information for the tumor growth study.

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Figure 1. Workflow of PBPK and PK model development. Gray box indicates data source for the model inputs, and blue box indicates model outputs.

Figure 2.

Whole body PBPK model for birinapant in mice. Organs are represented by rectangles and interconnected *via* blood flows Q . Symbols are defined in Table 2.

Figure 3. Whole body PBPK model for gemcitabine (dFdC) and its metabolite (dFdU) in mice. The interpretation of symbols and labels is the same as in Figure 1. Symbols are defined in Table 3.

Figure 4.

Plasma and tissue concentration-time profiles of birinapant following an IV bolus dose of 5 mg/kg in tumor-bearing mice. The *solid* circles are digitized observations from [18], the *solid black* lines are model-fitted profiles for MDA-MB-231 xenografts, and the *red dashed* lines are model simulations for concentration profiles in mice with PANC-1 pancreatic cancer xenografts.

Figure 5.

Plasma and tissue concentration-time profiles of gemcitabine (dFdC) and its metabolite (dFdU) following IV bolus doses of 0.1, 20, 30, and 160 mg/kg in mice. Symbols are digitized observations and lines depict model-fitted profiles. Data sources for observations are summarized in Table S-1 [5, 6, 16, 17].

Figure 6.

Observed and model-fitted tumor growth profiles for control and treatment groups in tumor-bearing mice. Gemcitabine was administered at 40 mg/kg once every three days for four doses, and the cycle was repeated beginning on Day 15 (q3d $\times$ 4 $\times$ 2); Birinapant was administered alone at 6, 20 or 60 mg/kg on a q3d $\times$ 10 schedule, or at 20 mg/kg on a q3d $\times$ 10 schedule in combination with 40 mg/kg gemcitabine on a q3d $\times$ 4 $\times$ 2 schedule. Symbols are measured values (mean $\pm$ SD, $n=10$) and the lines are model-fitted profiles.

Figure 7.

Prediction using the network model to link the PBPK and PD models. The same dosing schedule as in Figure 6 was applied. (a) Model-predicted tumor growth profiles for control and treatment groups in tumor-bearing mice. Signaling for gemcitabine-induced apoptosis (b) and DNA repair (c) when gemcitabine and birinapant were dosed either simultaneously or sequentially with birinapant dosed 24 h after gemcitabine.

Table 1.

Typical physiological parameter values for 25 g mice.

Tissue	Volume^a (ml)	Blood flow^a (ml/h)	Fraction of vascular space^c	<i>K_p</i> value^d (gemcitabine)
Plasma	1.23	840	1.0	
Lung	0.183	840	0.10	1.06
Spleen	0.0875	3.82 ^b	0.10	3.95
Bone marrow	1.45	92.4	0.053	N/A
Tumor	0.300	1.30 ^b	0.070	N/A
Kidney	0.418	76.4	0.10	2.05
Liver	1.38	135	0.10	1.09
Pancreas	0.150	7.64 ^b	0.10	1.44
Intestine	1.06	106	0.029	1.22
Muscle	9.60	134	0.019	1.12
Heart	0.125	55.4	0.053	1.22
Brain	0.413	27.7	0.080	0.569

a. Values obtained from Brown et al [21].

b. Values obtained from Pawaskar et al [22].

c. Values obtained from Baxter et al [20].

d. Values estimated based on $AUC(tissue)/AUC(plasma)$ from Shipley et al [5]. The K_p value for intestine is the average of stomach and small intestines. The K_p value for muscle and brain were not utilized due to the perfusion-rate limiting feature of the two organs. The K_p value for spleen was not utilized because concentrations in spleen are available.

Table 2.

Parameter estimates for birinapant pharmacokinetics. Kp_{tissue} is the partition coefficient, PS_{tissue} is the permeability surface area product, and CL_{liver} reflects the hepatic clearance.

Tissue	Parameter (Unit)	Estimate	CV%	Predicted Kp ^a
Lung	Kp_{lug}	99.3	26.4	11.1
Tumor (MDA-MB-231)	Kp_{tum1}	22.1	20.5	
Tumor (Pancreatic cancer)	Kp_{tum2}	2.00	Fixed	
Intestine	Kp_{int}	83.0	19.7	6.96
Liver	Kp_{liv}	138	26.5	12.8
Kidney	Kp_{kid}	195	26.2	14.1
Heart	Kp_{hrt}	11.0	23.8	6.46
Brain	Kp_{bra}	1.74	30.5	1.29
Carcass	Kp_{res}	73.9	25.8	
	CL_{liv} (ml/h)	105	20.6	
	PS_{tum} (ml/h)	0.0916	23.8	
	PS_{kid} (ml/h)	2.95	32.8	
	PS_{hrt} (ml/h)	0.206	37.3	
	PS_{bra} (ml/h)	0.00781	34.9	
	PS_{res} (ml/h)	19.0	22.9	

a. The Kp value predicted using the Rodgers et al method for moderate to strong bases [23].

Table 3.

Parameter estimates for gemcitabine pharmacokinetics. Kp_{tissue} is the partition coefficient, PS_{tissue} is the permeability surface area product, CL_{liver} reflects the hepatic clearance of dFdC, CL_{kidney} reflects the renal clearance of dFdC, and $CL_{dFdU,kidney}$ reflects the renal clearance of dFdU.

Tissue	Parameter (Unit)	Estimate	CV%
Tumor	Kp_{tum}	0.137	11.3
Spleen	Kp_{spl}	4.53	17.6
Bone Marrow	Kp_{bom}	0.498	16.2
Carcass	Kp_{res}	8.16	18.1
Lung	Kp_{lug}	1.06 ^a	Fixed
Kidney	Kp_{kid}	2.05 ^a	Fixed
Liver	Kp_{liv}	1.09 ^a	Fixed
Pancreas	Kp_{pan}	1.44 ^a	Fixed
Intestine	Kp_{int}	1.22 ^a	Fixed
Heart	Kp_{hrt}	1.22 ^a	Fixed
	CL_{kid} (ml/h)	14.7	39.6
	CL_{liv} (ml/h)	2.97	160
	PS_{spl} (ml/h)	0.275	27.4
	PS_{res} (ml/h)	26.2	9.49
Kidney (dFdU)	$Kp_{U,kid}$	1.25	30.4
Liver (dFdU)	$Kp_{U,liv}$	4.30	35.7
Remainder (dFdU)	$Kp_{U,res}$	0.664	39.4
	$CL_{U,kid}$ (ml/h)	7.10	25.9

a. The Kp value calculated from the $AUC(tissue)/AUC(plasma)$ from Shipley et al [5].

Table 4.

Pharmacodynamic parameter estimates for tumor growth.

Parameter (unit)	Annotation	Estimate	CV%	Confidence Interval (95%)
R_0 (mm ³)	Baseline tumor volume	197	4.50	
k_{ng} (h ⁻¹)	Net tumor growth rate	0.00571	4.85	
$Kmax_b$ (h ⁻¹)	Maximal rate of cell killing induced by birinapant	0.0167	110	
$KC50_b$ (ng/ml)	Birinapant concentration in tumor required for half maximum cell-killing effect	2.90×10^3	165	
$Kill_g$ (h ⁻¹ ·(ng/ml ⁻¹))	Rate of cell killing induced by gemcitabine	2.76×10^{-5}	8.63	
Ψ (on $KC50_b$)	Interaction term	1.27	14.9	[0.893, 1.65]
Ψ (on $Kill_g$)	Interaction term	0.944	3.51	[0.877, 1.01]