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The Effect of Loperamide and Fentanyl on the Distribution Kinetics of Verapamil in the Lung and Brain in Sprague Dawley Rats

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***Abstract*—**Verapamil has been shown to inhibit fentanyl uptake *in vitro* and is a potent P-glycoprotein inhibitor. Tissue partitioning of loperamide, a commercially available opioid, is closely controlled by the P-gp efflux transporter. The following studies were designed to evaluate the effect of opioids on verapamil partitioning in the lung and brain, *in vivo*. Opioid (fentanyl or loperamide) was administered by intravenous infusion to Sprague Dawley rats alone or in combination with verapamil and plasma, with lung and brain tissues were collected at 1, 5, 6, 8, 10 and 60 minutes. Drug dispositions were modeled by recirculatory pharmacokinetic models. Fentanyl slightly increased the verapamil lung (P_L) partition coefficient yet decreased the brain (P_B) partition coefficient. Furthermore, loperamide significantly increased $P_L$and P_B. Fentanyl reduced the verapamil volume of distribution (V_1) and verapamil elimination clearance (Cl_E). Fentanyl decreased verapamil brain partitioning, yet increased verapamil lung partitioning. Also, loperamide increased lung and brain partitioning *in vivo*. These results suggest that verapamil and fentanyl may be substrates of an unidentified inward transporter in brain tissue and confirm that verapamil and loperamide are substrates of the efflux transporter P-gp.

***Keywords*—**efflux transporter, elimination clearance, partition coefficient, verapamil.

I. INTRODUCTION

VERAPAMIL, a phenylalkylamine calcium channel antagonist, is widely used for the treatment of various cardiovascular disorders. Verapamil undergoes significant pulmonary uptake in the human lung with about 50% of the drug accumulating in lung tissue following intravenous bolus administration [1]. Many basic lipophilic amines such as the opioid analgesic fentanyl [2,3] are known to undergo significant, reversible, pulmonary uptake following intravenous administration. This extensive first-pass uptake of verapamil and fentanyl is thought to be due to the high lipid solubility of the drugs. However, it has recently been shown that pulmonary uptake of the opioid analgesic fentanyl is the result of a saturable specific uptake mechanism blocked by verapamil [4-6]. Additionally, we have demonstrated that cultured bovine brain microvascular endothelial cells also have a saturable energy-dependent process mediating the uptake of fentanyl perhaps by an unidentified inward transporter inhibited by verapamil [7]. Verapamil is also a substrate/inhibitor of the efflux transporter P-glycoprotein (P-gp). It has been used in many clinical trials for facilitating the transport of anticancer drugs across the blood brain barrier, which would otherwise be effluxed by P-gp. Recently we discovered that *in vivo*, verapamil inhibits fentanyl uptake in the brain, yet it inhibits the efflux of the P-gp mediated opioid, loperamide [18]. We hypothesized that fentanyl would reduce verapamil uptake in lung and brain, and that loperamide a P-gp substrate, would reduce clearance of verapamil in the lung and brain. Therefore, we studied lung and brain uptake, distribution and elimination of verapamil in the presence and absence of fentanyl and loperamide in Sprague Dawley rats.

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II. METHODS

Experimental Protocol

After approval from the University of Colorado health Sciences Center (UCHSC) Animal Care and Use Committee, adult male Sprague-Dawley rats weighing 300-350 g were purchased with indwelling cannulae (jugular venous catheters for drug infusion and a carotid artery catheter for blood collection) from Harlan (Madison Wisconsin). The rats were housed in the UCHSC animal facility. They were kept on a 12-hour light/dark cycle and were fed standard laboratory chow. A target concentration controlled infusion of verapamil hydrochloride (Abbott Laboratories, North Chicago, Illinois) was administered for five minutes at a target concentration of 1 mg/mL prior to initiation of opioid infusion and administration was continued for ten minutes (from time t= -5 to 10 min). The initiation of opioid infusion was defined as time 0 min of the experiment. Verapamil and opioids were infused *via* the jugular venous catheter. Target concentration controlled infusion was accomplished using a Harvard 22 syringe pump (Harvard Apparatus, Holliston, Massachusetts) that was controlled *via* a serial interface by a personal computer running the STANPUMP target concentration controlled infusion software (written by Dr. S.L. Shafer). The infusion software was assuming a 3-compartment

pharmacokinetic model for verapamil [8,9]. The animals received a 5-min intravenous infusion (from time t= 0 to 5 minutes) of either fentanyl citrate (Abbott Laboratories, North Chicago, Illinois) using an infusion rate of 5.25 μg/kg/min) or loperamide hydrochloride (Sigma Aldrich, St. Louis, Missouri) using an infusion rate of 0.475 mg/kg/min. Rats were euthanized by decapitation at -5, 5, 6, 8, 10, or 60 min (n= 4 per time point). Blood (5 mL) was collected from the carotid catheter prior to decapitation and drawn into tubes containing citrate as anticoagulant. Plasma was separated following a standard centrifugation protocol (400 g, 10 min, 4°C). The skull was opened immediately after decapitation and brain tissue was collected and flash-frozen in liquid nitrogen. Hereafter, a thoracotomy was performed to collect lung tissues that were also immediately frozen in liquid nitrogen. Plasma, lung, and brain tissues were stored at –80°C until analysis of drug concentrations using a validated high-performance liquid chromatography/ tandem mass spectrometry (LC-MS/MS) assay.

Analytical Methods

Prior to LC-MS/MS analysis, rat brain and lung (approximately 10 mg) were weighed and homogenized with 2 mL KH_2PO_4 buffer pH=7.4 (1M) using a teflon-glass manual homogenizer. Homogenized samples were stored at -80ºC before analysis. On the day of analysis, plasma, homogenized brain or lung were thawed on ice. Four hundred μL of a precipitation solution consisting of 0.06 M $ZnSO_4$ solution and methanol (3:7, v/v) as well as 10 μL of the internal standard were added to 200 μL of plasma or tissue homogenates. (±)-Methoxy verapamil hydrochloride (Sigma Aldrich) was used as internal standard. The final concentration in the extracted samples was 50ng/mL. After vortexing for 2 min, samples were centrifuged (13000 g × 5 min, 4°C) to remove precipitated proteins. One hundred μL of the supernatant was directly injected into the HPLC system (series 1100, Agilent, Waldbronn, Germany) using a Leap autosampler (CTC Analytics AG, Zwingen, Switzerland) equipped with a cool stack. Extracted samples were kept at +4°C in the autosampler.

Supernatants were loaded onto an extraction column (4.6 × 12.5 mm, 5 μm particle size, Eclipse XDB-C8, Agilent) and were washed with a high flow of 5 mL/min, 80% 0.1% formic acid/ 20% methanol for 1 min. The switching valve was then activated and the analytes were back-flushed onto a C8 analytical column (4.6 × 12.5 mm, 5 μm particle size, Eclipse XDB-C8, Agilent). A linear gradient was used for separation: methanol increased from 55% to 100% in 4 min and was kept at 100% methanol for 1 min. The flow rate was 1 mL/min. Column temperature was maintained at 40ºC.

An MDS Sciex API4000 triple-stage quadrupole mass spectrometer (Applied Biosystems, Foster City, California) was used as detector. The mass spectrometer as well as the HPLC was controlled and data was processed using the Analyst software (version 1.3., Applied Biosystems). The analytes eluted from the HPLC column into a turbo-ion spray source. Nitrogen was provided by a Zero Air Generator (Analytical Gas Systems) and Nitrogen (>99.999% purity) was used as collision activated dissociation (CAD) spray and curtain gas. Positive ions were monitored using Multiple Reaction Monitoring (MRM). During assay development, MRM parameters for each analyte were adjusted by directly infusing verapamil or its internal standard solution (0.1 μg/ mL, 80% methanol/ 20% 0.1% formic acid) into the electrospray source using a syringe infusion pump (KD Scientific, Holliston, Massachusetts). The following MRM parameters were found to give the best sensitivities: the source temperature was set to 480ºC and the ion spray voltage was 5000V. Gases were adjusted to 20 for nebulizer gas, 15 for turbo gas, 15 for curtain gas and 8 for CAD gas (all arbitrary units as used in the Analyst software). The declustering potential was set to 50V. The dwell time for each transition was 200 ms. Data was collected and the major product ion transitions were monitored: verapamil m/z= 455.6 → 165.6 and m/z= 485.6 → 333.5 for the internal standard (±)-methoxy verapamil.

III. Data Analysis

The pharmacokinetics were analyzed using the SAAM II software (SAAM Institute, Seattle, Washington) using a naive pooled-data technique. Model parsimony was tested using the Akiake information criterion (AIC) [10]. The data from the verapamil infusion alone and verapamil infusion with either fentanyl or loperamide treatment were lumped into separate models with linked parameters. Plasma kinetics were modeled with a one-compartment open model in which volume of distribution (V_1) and elimination clearance (Cl_E) were fit to the plasma verapamil concentration-time data. Data fit with this model had lower AIC than that fit with a two-compartment model.

To test the hypothesis that either fentanyl or loperamide affects the plasma pharmacokinetics of verapamil, all data were fit to a unified pharmacokinetic model for verapamil alone. Each of the adjustable parameters (V_1, Cl_E) was then fit so that each parameter could have a covariate parameter for the experimental condition in which either fentanyl or loperamide was infused. To test whether a covariate for the fentanyl or loperamide condition produced a statistically significant change in the pharmacokinetic parameter, the AIC with the added covariate in the model was compared to the AIC in the simpler model without the covariate. The model with the lowest AIC was then chosen as shown in table 1and 3.

Brain and lung concentration-time data were modeled by adding partition coefficients P_B and P_L to describe the ratio between plasma verapamil concentrations and those in brain and lung, respectively. To test the hypothesis that either fentanyl or loperamide changes plasma: tissue partitioning in the brain and lung, all data were first fit to a unified pharmacokinetic model for verapamil alone and then each of the plasma: tissue partition coefficients (P_B, P_L) were fit so that each coefficient could have a covariate parameter for the experimental condition in which either fentanyl or loperamide was infused. To test whether a covariate for the fentanyl or loperamide condition produced a statistically significant change in the partition coefficient, the AIC with the added covariate in the model was compared to the AIC in the simpler

model without the covariate. Model 7 had the lowest AIC as shown in table 1and 3.

IV. Results

Effect of fentanyl on verapamil pharmacokinetics. Rat arterial plasma, lung and brain verapamil concentrations in the absence and presence of fentanyl versus time relations were well-characterized by the model from the moment of injection. Visual comparison of the measured and predicted verapamil concentration versus time relationships revealed no model misspecification (Figures 1, 2).

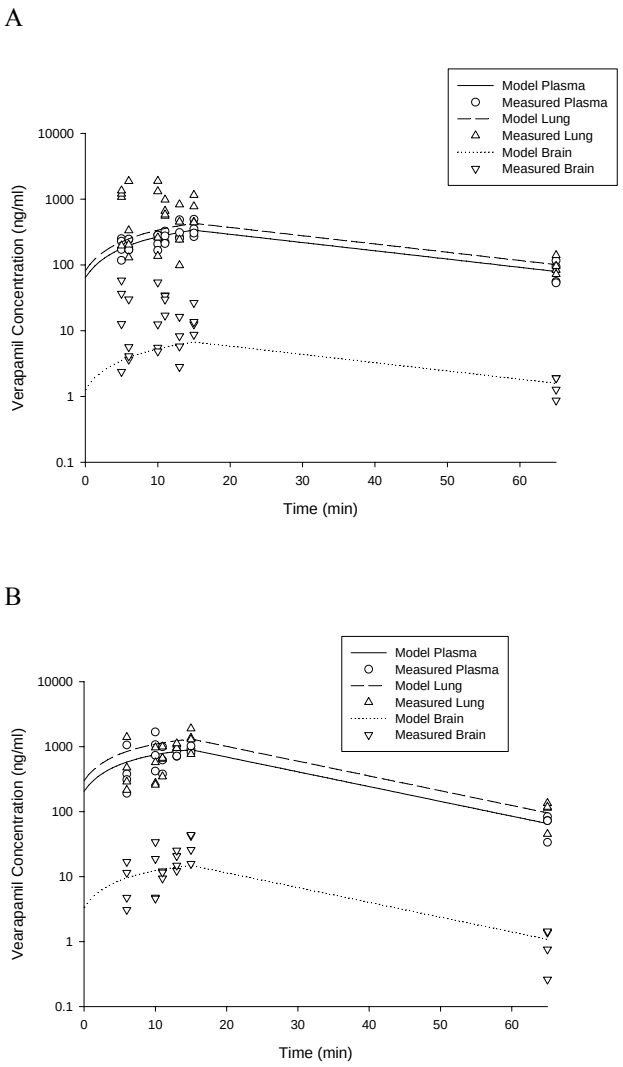

Fig. 1. Verapamil concentration in Sprague Dawley rats arterial plasma, lung, and brain verapamil only (A), with fentanyl (B). The symbols represent drug concentrations, whereas the lines represent concentrations predicted by the model. Predicted plasma drug concentrations (solid line), measured plasma drug concentration (opened circle), predicted lung drug concentrations (dashed line), measured lung drug concentration (triangle up), predicted brain drug concentration (dotted line), measured brain concentration (triangle down).

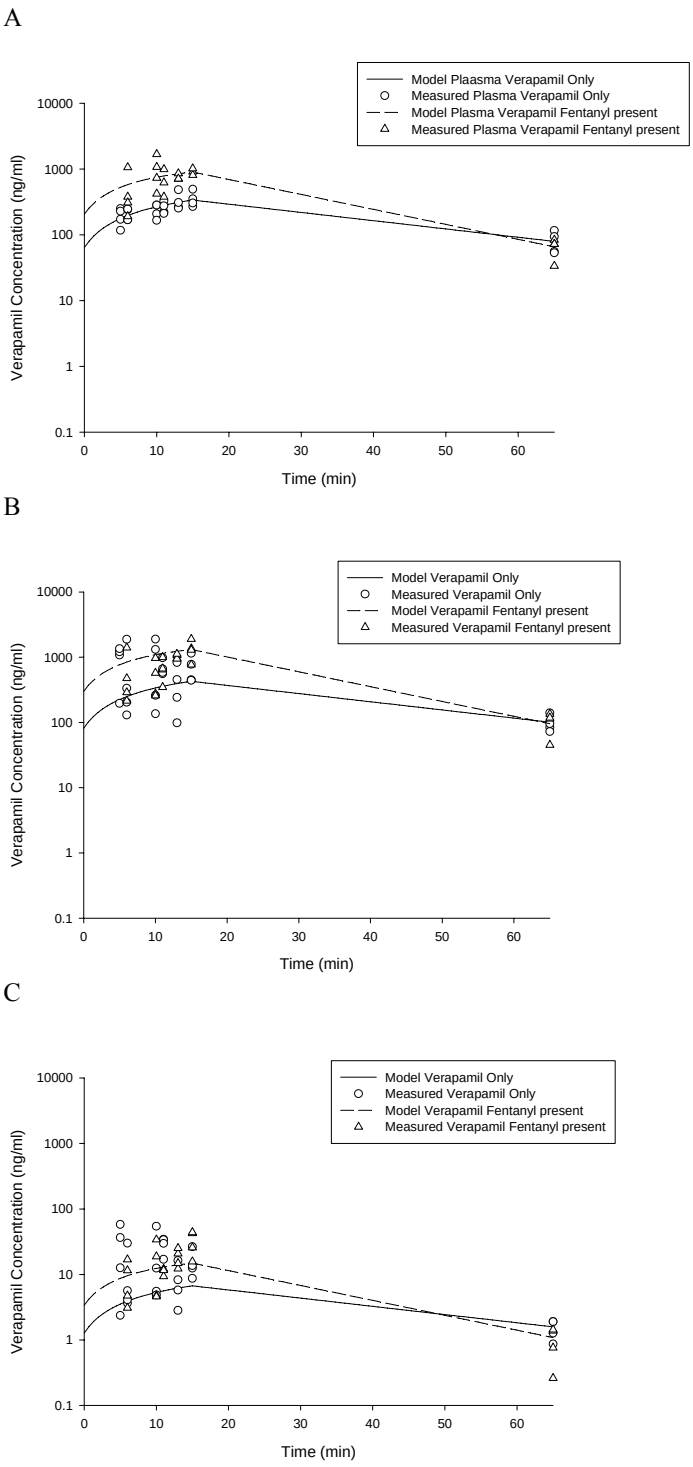

Fig. 2. Verapamil concentration in Sprague Dawley rats arterial plasma (A), lung (B), and brain (C), in the absence and the presence of fentanyl. The symbols represent drug concentrations, whereas the lines represent concentrations predicted by the model. Predicted drug concentrations in theabsence of fentanyl (solid line), measured drug concentration in the absence of fentanyl (circle), predicted drug concentrations in the presence of fentanyl (dashed line), measured drug concentration in the presence of fentanyl (triangle).

The pharmacokinetic parameters of verapamil were described by a one-compartment model with elimination clearance (Cl_E) from the central compartment, blood partition coefficients for lung (P_L), and brain (P_B) and volume of distribution (V_1). In the presence of the centrally acting opioid, fentanyl, the same one-compartment model applied but fentanyl covariates were added to the parameters (Table 1).

TABLE I MODEL FOR VERAPAMIL IN THE PRESENCE OF FENTANYL

	Model 1 Control plasma	***Model 2*** CL_E	***Model 3**** CL_E $+V_1$	***Model 4*** Control plasma+ lung +brain	***Model 5*** P_B	***Model 7**** P_{B+} P_L
Verapamil+ Fentanyl	3.08 X 10^{-3}	1.6103 X 10^{-3}	1.548 X 10^{-3}	5.437 X 10^{-3}	5.424 X 10^{-3}	5.408 X 10^{-3}

* Model selected for verapamil in the presence of fentanyl

The results indicated that fentanyl reduced V_1 and Cl_E by 68% and 40% respectively also P_B decreased by 16 % yet P_L increased by 22 % (Table 2).

TABLE II PHARMACOKINETIC VARIABLES FOR VERAPAMIL IN THE ABSENCE AND THE PRESENCE OF FENTANYL OR LOPERAMIDE (N = 4)

Treatment	P_B	P_L	Cl_E ml/kg/min	V_1 ml
Verapamil	0.020	1.27	0.051	1.77
Verapamil + Fentanyl	0.017	1.46	0.029	0.55
Verapamil + Loperamide	0.063	2.4	0.048	1.81

P_B = blood/brain partition coefficient
P_L = blood/lung partition coefficient
Cl_E = elimination clearance
V_1 = volume of distribution

Effect of loperamide on verapamil pharmacokinetics. Rat arterial plasma, lung and brain verapamil concentration in the absence and presence of loperamide versus time relations were well-characterized by the model from the moment of injection. Visual comparison of the measured and predicted verapamil concentration versus time relationships revealed no model misspecification (Figures 3, 4).

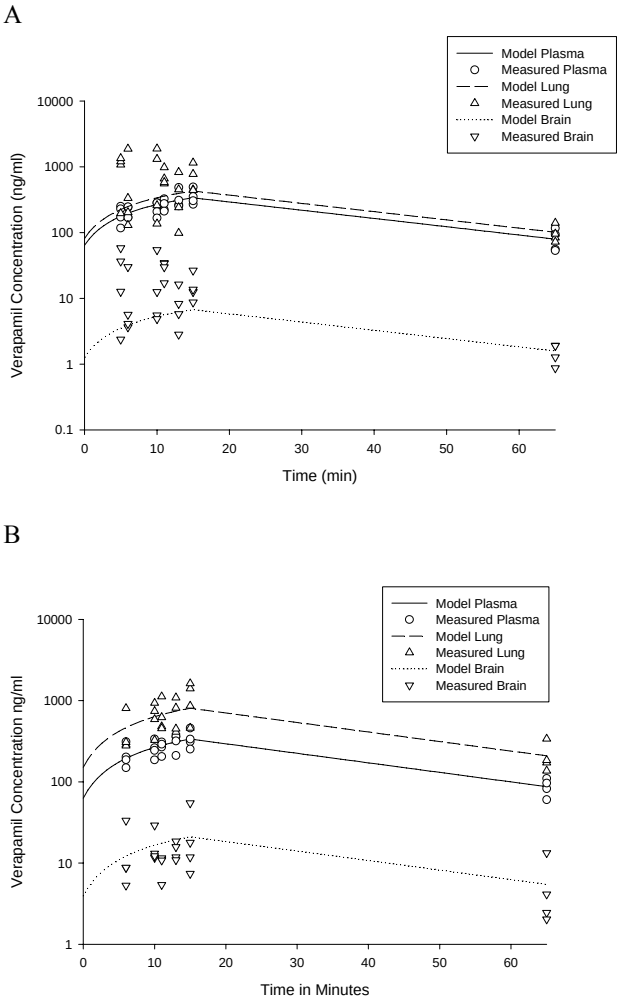

Fig. 3. Verapamil concentration in Sprague Dawley rats arterial plasma, lung, and brain verapamil only (A), with loperamide (B). The symbols represent drug concentrations, whereas the lines represent concentrations predicted by the model. Predicted plasma drug concentrations (solid line), measured plasma drug concentration (opened circle), predicted lung drug concentrations (dashed line), measured lung drug concentration (triangle up), predicted brain drug concentration (dotted line), measured brain concentration (triangle down).

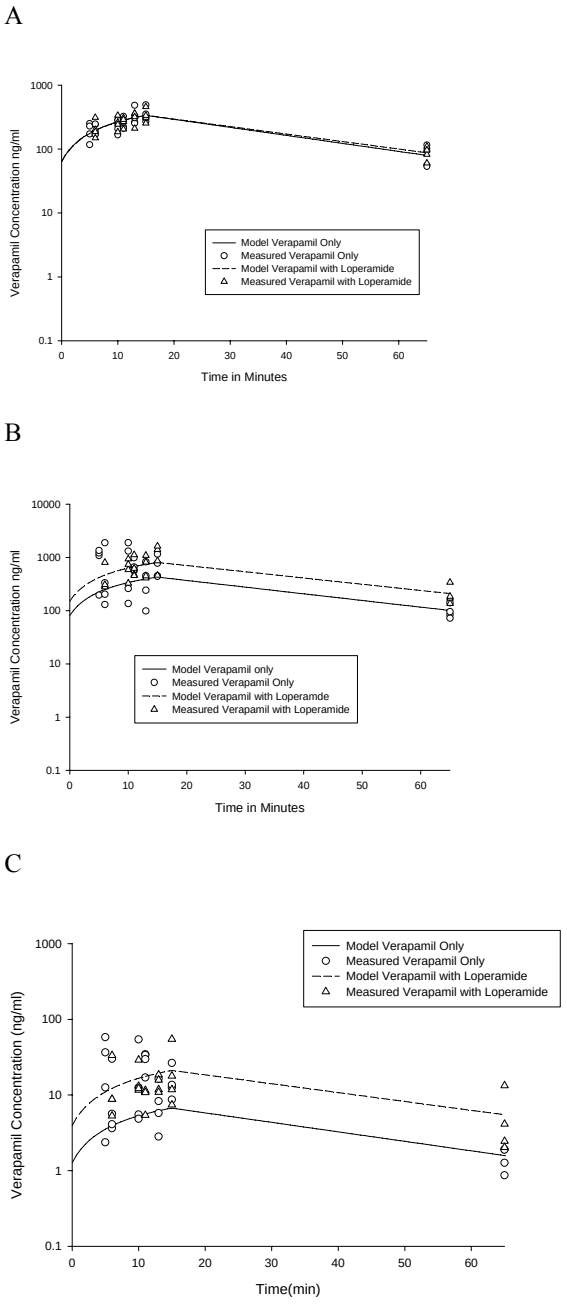

Fig. 4. Verapamil concentration in Sprague Dawley rats arterial plasma (A), lung (B), and brain (C), in the absence and the presence of loperamide. The symbols represent drug concentrations, whereas the lines represent concentrations predicted by the model. Predicted drug concentrations in the absence of loperamide (solid line), measured drug concentration in the absence of loperamide (circle), predicted drug concentrations in the presence of loperamide (dashed line), measured drug concentration in the presence of loperamide (triangle).

The pharmacokinetic parameters of verapamil were described by a one-compartment model, with elimination clearance (Cl_E) from the central compartment, blood partition coefficients for brain (P_B) and lung (P_L), and volume of distribution (V_1) In the presence of the 'peripherally' acting opioid, loperamide, the same one-compartment model applied but loperamide covariates were added to the parameters (Table 3).

TABLE III MODELS FOR VERAPAMIL IN THE PRESENCE OF LOPERAMIDE

	Model 1 Control plasma	***Model 2*** CL_E	***Model 3†*** CL_E $+V_1$	***Model 4*** Control plasma+ lung +brain	***Model 5*** P_B	***Model 7†*** P_{B+} P_L
Verapamil+ loperamide	4.536 X 10^{-3}	4.423 X 10^{-3}	4.422 X 10^{-3}	1.744 X 10^{-3}	1.743 X 10^{-3}	1.169 X 10^{-3}

† Model selected for verapamil in the presence of loperamide.

The results demonstrated that in the presence of loperamide, P_B and P_L increased by 3.1and 1.8 fold respectively Cl_E and V_1were unchanged (Table2).

V. DISCUSSION

We wished to measure the distribution of verapamil and to determine whether there may be differences in their transport in to the lung and brain of rats in the presence of fentanyl and loperamide. In addition, we wished to evaluate the pharmacokinetics of verapamil using a high resolution recirculatory model. This will allow us to carefully examine any possible differences in disposition that could reasonably be attributed to the presence of the two opioids.

Our results indicated that fentanyl reduced V_1, Cl_E and verapamil P_B while P_L increased in Sprague Dawley rats and loperamide increased P_B and P_L. These results suggest that verapamil uptake in the rat brain is inhibited in the presence of fentanyl, yet there is an increase in uptake in lung in the presence of verapamil *in vivo*. Furthermore, loperamide increased verapamil brain and lung uptake. Essentially, in the presence of fentanyl verapamil brain concentrations decreased while verapamil lung concentrations increased. Loperamide increased verapamil brain and lung concentrations.

Previously, we showed that uptake of verapamil and fentanyl in the brain occurred via an unidentified inward transporter[7]. It is not entirely surprising that, like fentanyl, verapamil is the substrate of a transporter directed inwardly across the endothelium. Both drugs are lipophilic basic amines, a fact that may underlie a shared substrate specificity. Both are classic examples of drugs demonstrating high pulmonary uptake [1,2]. Recently, organic anion transporter polypeptides were found in endothelial cells lining capillaries in the brain and lung of rats (Oatp) and humans (OATP)[11,12]. A number of the Oatp/OATP transporters appear to have shared drug substrates with the efflux transporter P-gp [13]. Verapamil and fentanyl are known substrates of the P-gp efflux transporter [7], yet the present study showed that fentanyl decreased verapamil partitioning in the brain of rats suggesting that verapamil and fentanyl may be substrates of an inward transporter in rat brain tissue, possibly Oatp. Further work is needed to establish whether or not one of the Oatp/OATP transporters is responsible for verapamil uptake in brain.

In contrast to our hypothesis, fentanyl increased verapamil partitioning in the lung. Also, verapamil and fentanyl are known substrates of the P-gp efflux transporter[7]. The results

demonstrate that verapamil concentrations increased in the Sprague Dawley rat lung; potentially caused by fentanyl inhibition of the outward P-gp mediated transport of verapamil. The present study suggests that P-gp may play an important role in verapamil pulmonary uptake.

The efflux transporter, P-glycoprotein, efficiently extrudes loperamide from brain and lung [14-16] and verapamil is a known substrate/competitive inhibitor of P-gp [16,17]. Our data indicating that loperamide significantly increased verapamil partitioning in lung and brain, confirms the findings of others that verapamil and loperamide are substrates of the outwardly directed P-gp. Furthermore, we have demonstrated that in the presence of loperamide there was a 3.3-fold increase in brain uptake of verapamil, and a 1.8 -fold increase in lung uptake. These data suggest that P-gp plays an important role in the brain uptake kinetics for intravenous verapamil.

Because this study was designed to give opioids for five minutes only and to measure lung and brain concentrations at 1, 5, 6, 10, and 60 minutes, further research is needed in which opioids are administered continuously with verapamil for fifteen minutes. Also, a greater number of sampling points between 10 and 60 minutes are needed to clearly delineate the pharmacokinetics of these drug interactions.

In conclusion, fentanyl decreased verapamil partitioning in the brain, yet increased verapamil partitioning in the lung, *in vivo*. Additionally, loperamide increased verapamil lung and brain partitioning. These observations suggest that verapamil and fentanyl may be substrates of both inward and outward transporters in brain tissue and confirm that verapamil and loperamide are substrates of the efflux transporter P-gp.

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