Pharmacokinetics of fentanyl in epileptic patients during concomitant therapy with phenytoin or carbamazepine

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Abstract

Fentanyl was first approved for clinical use in the United States in 1968, and is an opioid used for pain medication and anesthesia. Clinical studies have shown that epileptic patients treated chronically with certain antiepileptic drugs, including carbamazepine and phenytoin, have higher fentanyl requirements during maintenance of anesthesia. The hypothesis is that antiepileptic drugs induce the activity of cytochrome P450 enzymes, causing increased hepatic clearance of fentanyl in epileptic patients. To characterize whether the pharmacokinetic of fentanyl are altered in patients receiving carbamazepine, 19 patients were recruited into this study. 11 patients were treated with carbamazepine based on their needs, and 8 patients served as controls. All patients received 200 mcg of fentanyl as a single intravenous bolus at anesthesia induction. Blood samples were collected at various time points for up to 9 hours. After liquid-liquid extraction by methyl tert-butyl ether, fentanyl concentrations in plasma samples were determined by UPLC-MS/MS. Concentration-time curves were fitted to a 2-compartment model or a 3-compartment model. Statistical analysis showed that mean fentanyl clearance was higher in patients receiving carbamazepine (20.1 mL/kg/min vs 13.2 mL/kg/min). We did not measure hepatic blood flow in this study but hepatic blood flow is known to be increased by enzyme–inducing drugs. Therefore the increased clearance of fentanyl in carbamazepine-treated patients is likely attributable to induction of hepatic drug-metabolizing enzyme activity together with an increase in hepatic blood flow.

Acknowledgements

I would like to thank my thesis advisor Dr. David J Greenblatt for his instruction on my projects. He is a gentle man with intelligence and patience. He also provided me with many suggestions about my PhD applications and future career plans.

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I would also like to thank Dr. Alexei Degterev for his instruction in my second rotation, and Dr. Emmanuel Pothos for being the second reader for my master thesis.

Lastly, I would like to express my gratitude to my parents, my uncle, and my aunt for their selfless love and unconditional support during my master's degree study.

Sincerely,

Zhijun Ma

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Chapter 1: Introduction

Fentanyl is a strong opioid (approximately 75–100 times more potent than morphine) which is highly lipophilic and extensively bound to plasma proteins [1]. It is commonly used in pain management and anesthesia owing to its high lipid solubility and potency [2]. Fentanyl exerts its pharmacological effect via its interaction with the μ-opioid receptor. The volume of distribution of fentanyl is large (3.5–8 L/kg) and its clearance relatively high (30–72 L/h) [3]. When fentanyl is given as an intravenous bolus, it is rapidly distributed from plasma into highly vascularized compartments. After uptake in the systemic circulation, redistribution to muscle and fat tissue occurs. Elimination half life is highly variable in various studies (219–853 min), particularly due to this redistribution [4–7].

Fentanyl is mainly metabolized in the liver by the cytochrome P450 iso-enzyme 3A4 (CYP3A4)-mediated N-dealkylation, resulting in the inactive metabolite norfentanyl [8-10]. Less than 1% is metabolized by alkyl hydroxylation, N-dealkylation, or amide hydrolysis to the inactive compounds hydroxyfentanyl, hydroxynorfentanyl and despropionylfentanyl. The inactive metabolites, and approximately 10% of the intact molecule, are mainly excreted by the kidneys [11–13]. However, a recent study showed that the CYP3A4- mediated N-dealkylation step may not be as important as previously thought. Unknown metabolic routes may be responsible for a significant part of fentanyl metabolism [14].

Carbamazepine is an antiseizure drug used in the treatment of epilepsy. Carbamazepine is also used as analgesic in trigeminal neuralgia, and may be used in the treatment of bipolar disorder [15, 17]. It works by blocking voltage-dependent sodium channels present on neuronal cell membranes. In other words, carbamazepine stabilizes the sodium channel in the inactivated state, leaving fewer channels available to open. This process prolongs the inactivated state of the channel, and inhibits the rapid and repetitive generation of action potentials in the epileptic focus [16,18].

Carbamazepine is mainly metabolized in the liver by the cytochrome P-450 (CYP) system. The major metabolite is carbamazepine-epoxide, which has anticonvulsant activity of uncertain significance. CYP3A4 is the main enzyme involved in the metabolism of carbamazepine; a lesser role is played by CYP2C8 and possibly CYP3A5. Minor metabolic pathways include multiple CYP enzymes, such as CYP2B6[19]. Carbamazepine stimulates transcriptional upregulation of CYP3A4 and other genes involved in its own metabolism. In addition, there are many drug-drug interactions with carbamazepine, because numerous drugs have been shown to induce or inhibit CYP3A4, or are metabolized by CYP3A4[20]. Therefore, when carbamazepine is given with drugs that can decrease or increase carbamazepine levels, close monitoring of carbamazepine levels is indicated and dosage adjustment may be required.

Patients receiving one or more anticonvulsant drugs for the treatment of seizures often receive fentanyl to relieve pain. Clinical studies showed that epileptic patients treated chronically with certain antiepileptic drugs (AEDs, carbamazepine, phenytoin and

valproic acid) have higher fentanyl requirement during maintenance of anesthesia [21]. Various pharmacokinetic and pharmacodynamics mechanisms could explain the observed resistance to fentanyl seen with anticonvulsants. Since the primary route of fentanyl clearance is N-dealkylation to norfentanyl, and this step is catalyzed predominantly by cytochrome P450 3A4 (CYP3A4), the enhanced hepatic clearance of fentanyl might be caused by induction of the microsomal enzyme cytochrome P450 (CYP450) system activity by carbamazepine [22].

This study aims to characterize if pharmacokinetics plays a role in the altered requirement for fentanyl in patients receiving chronic AED treatment. Better understanding of the pharmacokinetics of fentanyl will guide the clinicians in applying the optimal dose for individual patients. This information is essential to prevent under dosing or overdosing of this important opioid, both of which have serious unwanted effects.

Chapter 2: Materials and Methods

2.1 Chemicals and materials

Fentanyl (l mg/mL in methanol) and fentanyl-d5 (100 µg/mL in methanol) were obtained from Cerilliant™ (Austin, TX), with a stated a minimum purity of 99%. LC-MS grade acetonitrile, water, and HPLC grade methanol were obtained from Fisher Scientific™ (Pittsburgh, PA). The Liquid-liquid extraction solvent methyl tert-butyl ether was from Fisher Scientific™ (Waltham, MA). All solvents were 99.9% purity as stated by the manufacturers. Water for the preparation of the mobile phase was drawn from a Milli Q TM filter (Millipore, Bedford, MA) apparatus.

2.2 HPLC instrumentation and Chromatographic conditions

Stability, recovery, and accuracy studies were performed using an Agilent 1200 series isocratic HPLC with Nova-Pak C18 4 µm 3.9x150 mm column. The UV detection wavelength is 215 nm. The mobile phase consists of a mixture of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), with a flow rate of 1.0 mL/min and run time of 10 min. The injection volume is 10 µL.

2.3 UPLC-MS/MS instrumentation

The analytic instrument is a ABSciex API 5000 triple quadrupole mass spectrometer equipped with a QJet ion guide and accelerated by a LINAC collision cell (AB Sciex, Foster City, CA) with an atmospheric pressure chemical ionization probe in a Turbo V ion source interfaced with a Waters Corporation (Milford, MA) Acquity ultra pressure liquid chromatograph. Analyst software 1.6.2 (AB Sciex, Foster City, CA) is used for

system control and data processing. The LC system is equipped with an Acquity UPLC HSS T3 1.8 μm, 2.1 X 50 mm HPLC column, and an Acquity UPLC HSS T3 1.8 μm VanGuard pre-column (Milford, MA).

2.4 Preparation of stock solutions

Standard stock solutions of fentanyl and fentanyl-d5 were prepared in methanol at concentrations of 50 μg/mL, respectively. Stock solutions were stored at 4°C until they were used for preparation of working solutions by adding appropriate volumes of mobile phase (acetonitrile and 0.5% formic acid solution, 85:15, v/v) for fentanyl, and methanol for the IS. Working solutions at different concentrations were prepared from above-mentioned stock solution freshly before use.

2.5 Calibration standards and quality control samples

Drug-free control human plasma was thawed at room temperature. Working solution of fentanyl were prepared in methanol at 100, 1000, 10000, 100000 pg/mL from stock solution. Calibration curves were prepared in the blank plasma spiked with the above working solution to produce the standard curve points equal to 10, 30, 100, 500, 1000, 8000, 15000, 20000 pg/mL or 100, 250, 500, 1000, 2500, 5000, 1000, 20000 pg/mL of fentanyl. In each standard solution, 5 ng/mL of fentanyl-d5 was used as internal standard.

2.6 Method validation

2.6.1 Linearity and sensitivity

Calibration curves were generated by using the ratios of the analytes peak area divided by the IS peak area versus concentration, and were fitted to the equation y=kx by weighted least-squares linear regression.

2.6.2 Accuracy and precision

The intra-batch precision and accuracy was determined by analyzing six sets of spiked plasma samples of fentanyl at each QC level (500, 1000, 5000 pg/mL in plasma) in a batch. The concentration of each samples was calculated using the standard curve prepared and analyzed on the same day. The post-preparative stability was measured by determining QC samples kept at the room temperature for 24 h.

2.6.3 Recovery (extraction efficiency)

To determine the extraction recoveries, two different sets of three concentrations of fentanyl (0.5, 5, 50 μg/mL fentanyl in blank plasma and 0.5, 5, 50 ng/mL fentanyl in mobile phase) were prepared. Samples are reconstituted with mobile phase after extraction. Sample extraction techniques such as protein precipitation and liquid-liquid extraction were all attempted. A preliminary evaluation of different extraction solvents such as tert butyl methyl ether (tBME), ethyl acetate, hexane, and toluene was conducted. The extraction recoveries were determined by comparing the peak area of samples spiked before extraction with those standard solutions in mobile phase at three different concentration level.

2.6.4 Protein precipitation

To 0.2 mL of a plasma sample, 1.0 mL of acetonitrile was added. After vortex-mixing for 1 min, the sample was centrifuged at 16000 × g at room temperature for 5 min. The supernatant was transferred to a clean tube and was evaporated to dryness. The residue was reconstituted with 0.2 ml methanol.

2.6.5 Liquid-liquid extraction

To 0.2 mL of a plasma sample, 1 mL of organic solvents (ex, tert butyl methyl ether, ethyl acetate, hexane, and toluene) was added. After vortex-mixing for 1min, the samples was centrifuged at 16000 × g at room temperature for 5 min. 800 mL of upper organic layer was transferred into a clean tube and then was evaporated to dryness. The residue was reconstituted with 0.2 mL mobile phase.

2.7 Stability study

The QC plasma samples were tested on the HPLC and then kept at room temperature for 24 h, extracted and then analyzed for a short-term stability study. The stock solution stability of fentanyl and the IS were evaluated by analyzing their working solutions kept at room temperature for 24 h, respectively.

2.8 Application of the assay

Plasma fentanyl concentrations are determined by liquid chromatography tandem mass spectrometry (LC-MS/MS). Liquid-liquid extraction was chosen for the sample preparation. All frozen human plasma samples were thawed at room temperature for 10

min. 200 μL of patient plasma sample was extracted with 1 mL methyl tert-butyl ether after addition 10 μL of IS solution (100 ng/mL fentanyl-d5 in methanol). The mixture was vortexed for 20 s, and then centrifuged at 16000 x g for 5 min. 800 μL of the upper organic layer was transferred and evaporated to dryness. The residue was reconstituted in 200 μL mobile phase for UPLC-MS/MS or HPLC-UV analysis.

Chapter 3: Results

3.1 UPLC-MS/MS optimization

To achieve symmetric peak shape and short run time for the analysis of fentanyl and fentanyl-d5, the mobile phase consisted of a mixture of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), with a flow rate of 0.5 mL/min and a run time of 2 min. Solvents A and B were combined in a gradient: 0-1 min: 85% A, 1-1.5 min: 50% A, 1.6-2 min: return to initial conditions and hold until 3 min. The mass spectrum analysis was conducted by electrospray ionization. Both the positive mode and the negative mode were used in the study and the result showed that the response of the positive ions was stronger and much more sensitive than negative ions. Hence, the positive mode of the mass spectrum was used in the following experiments. In the Q1 precursor full scan spectra, the m/z 337 was the most abundant ions of fentanyl was and m/z 342 for fentanyl-d5. The product ions scan spectra was investigated using the product ion mode, the most abundant ions were m/z 95 for fentanyl and 95 for fentanyl-d5. Other mass spectrometry parameters such as spray voltage, capillary temperature, collision energy, curtain gas, and collision gas were optimized by continuously injecting 500 ng/ml fentanyl or 500 ng/ml fentanyl-d5. The auto-tuning functions of Absciex MS system were incorporated into the tuning process to achieve quick and reproducible optimization of mass spectrometric conditions for different compounds. To maximize precursor ion intensity and to identify their product ion intensity, the auto-tuning function offered by the Analyst was used, leading to an immediate generation of optimized instrument parameters for the analytes. The electrospray source is operated in the positive ionization mode, using collision gas (CAD)

12, curtain gas (CUR) 20, ion source gas 40 and ion spray voltage 5500 V with temperature 500°C. The instrument is operated in the multiple reaction monitoring (MRM) mode. The following MRM transitions of precursor ions to product ions were selected: fentanyl, m/z 337.2→188.2 (collision energy, CE 24 V); fentanyl-D5, m/z. 342.2→l88.2 (collision energy, CE 35 V). The scan time is 100 ms for all analytes. The Declustering Potential (DP) is 125 mV for fentanyl and fentanyl-d5. The Entrance Potential (EP) is 10 mV for fentanyl and fentanyl-d5. Collision Cell Exit Potential (CXP) is 16 mV for fentanyl and fentanyl-d5. The positive ion spectrum of fentanyl and fentanyl-d5 and the chromatogram are shown in Figure 3.1 and Figure 3.2.

Figure 3.1: The positive ion ESI-MS/MS spectrum for fentanyl and fentanyl-d5

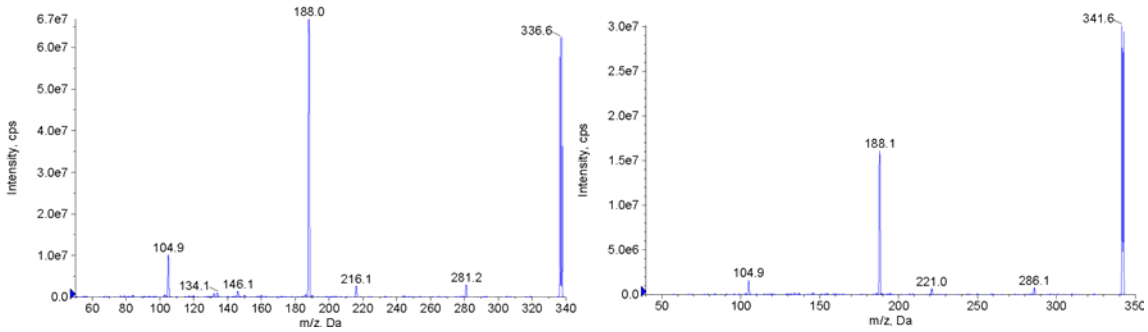
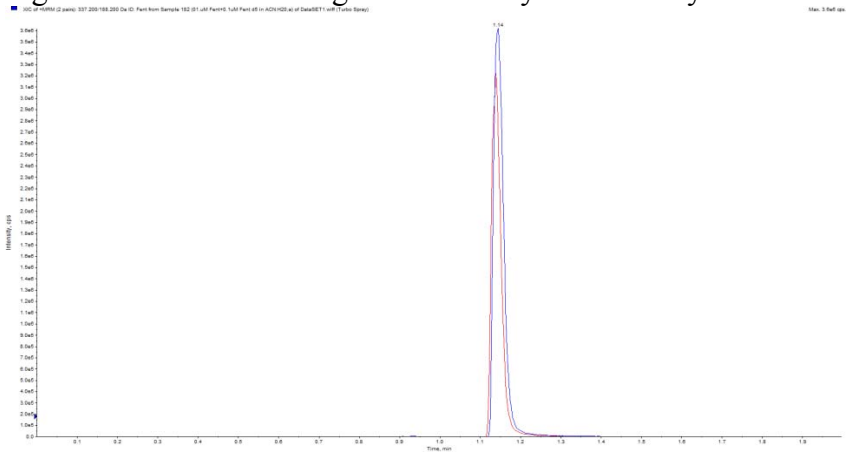


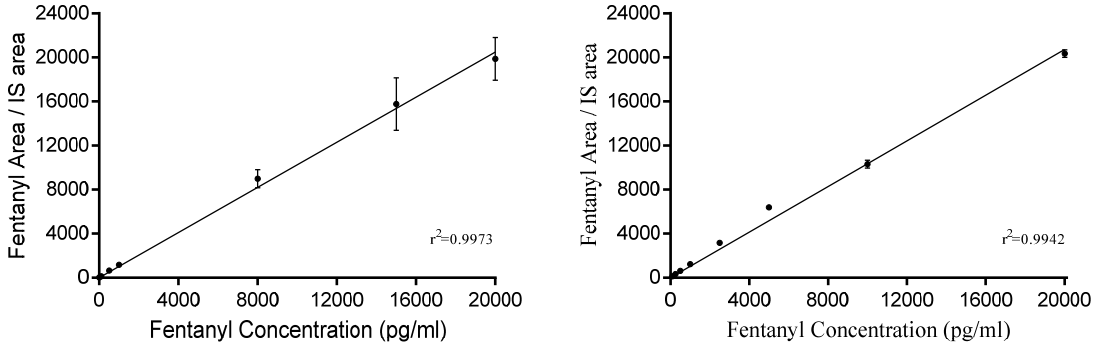
Figure 3.2: The chromatogram of fentanyl and fentanyl-d5



3.2 Linearity of calibration curve

The method showed a good linear response over the selected range of 10 to 20,000 pg/ml by weighted ($1/x^2$) least squares linear regression analysis. The mean standard curve was typically described by the equation: y=1.0274x, r^2=0.9973, where x corresponds to the peak area ratio of fentanyl to the IS and y refers to the concentration of fentanyl added to the plasma. For the 100 to 20,000 pg/ml concentration range, the mean standard curve was typically described by the equation: y=1.0356x, r^2=0.9942. Results of five representative standard curves for LC-MS/MS determination of fentanyl are given in Figure 3.3. The lower limit of quantification (LLOQ) for fentanyl is 0.01 ng/ml and upper limit of quantification (ULOQ) for fentanyl is 20 ng/ml.

Figure 3.3: Calibration Curve of fentanyl
(Left calibration cuve:10 pg/ml to 20,000 pg/ml; Right calibration cuve:100 pg/ml to 20,000 pg/ml)



3.3 Precision and accuracy

QC samples containing low, medium and high fentanyl concentrations in plasma were used to evaluate accuracy and precision of the developed assay method. The intra-day assay accuracy and precision were determined by analyzing replicates (n=6) of the QC

samples prepared on the same day. Data for precision are presented in Table 3.1. The assay accuracy was expressed as a percentage of the nominal concentration (observed concentration divided by nominal concentration) × 100%, and the precision was expressed by the coefficient of variance (CV).

Table 3.1
The precision and accuracy of the method for determining fentanyl in human plasma

Concentration added (ng/mL)	Intra-batch (n=6)		
	Concentration found (mean ± SD, ng/mL)	Accuracy (%)	Precision (%)
0.1	0.10 ± 0.01	3.98 ± 3.57	15.56
1	1.03 ± 0.02	3.00 ±2.16	4.85
5	5.22 ± 0.38	7.37 ± 4.86	7.77

3.4 Extraction efficiency

The extraction recovery was determined by comparing the peak area obtained after extraction with the peak area obtained without extraction. The mean extraction recoveries of fentanyl are shown in Table 3.2. In plasma samples, protein precipitation recoveries at 5 and 50 μg/mL were 54.4% and 57.8%, extraction recoveries in acetyl acetate were 53.5% and 56.7%, while in tert butyl methyl ether, extraction recoveries were 96.8% and 104.7%. In toluene, the extraction ratio extraction recoveries were 53.5% and 56.7%, while in hexane, the extraction ratio extraction recoveries were 67.7% and 74.7%. As a result, liquid-liquid extraction by tert butyl methyl ether was used for extraction.

Table 3.2
The extraction efficiency of the method for determining fentanyl in human plasma

	Concentration (μg/mL)	Recovery of extraction (%)
Liquid-liquid extraction		
Tert butyl methyl ether	5	96.8%
	50	104.7%
Toluene	5	53.5%
	50	56.7%
Hexane	5	67.7%
	50	74.7%
acetyl acetate	5	46.1%
	50	53.7%
Protein precipitation	5	54.4%
	50	57.8%

3.5 Stability

The stability experiments were conducted to evaluate the stability of fentanyl in stock solutions and in plasma samples at room temperature for 24 h. Each stability test in plasma included five replicates of three different concentrations. (5 and 50 μg/mL) of QC samples. The stability of spiked QC samples were compared with freshly prepared quality control samples. The results obtained were well within the acceptable limits. The result showed the reliable stability behavior of fentanyl under the tested condition.

Chapter 4: Clinical study

4.1 Protocol and institutional review

This study was performed in collaboration with Drs. Ala Nozari and J. A. J. Martyn of Massachusetts General Hospital and the Shriners Burns Institute. The study protocol and consent document were reviewed and approved by the Institutional Review Board serving those institutions. All study participants provided written informed consent prior to initiation of the study.

4.2 Subjects and procedures

A total of 19 subjects (11 females and 8 females) participated in this study. 2 subjects in control groups were withdrawn due to personal reasons. Table 4.1 shows patients characteristics in the study. Most patients in the study are Caucasians with one African American. The average dose of carbamazepine is 720 mg/day for each patient that received carbamazepine. The serum creatinine level is within the normal range in all the patients, indicating normal kidney function.

4.3 Pharmacokinetic analysis

A pharmacokinetic two-compartment model or three-compartment model was used to fit the data. The corresponding two equation are $C = Ae^{-\alpha t} + Be^{-\beta t}$ or $C = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$, where the C corresponds to the drug concentration in the plasma at different times[23]. The equation is fitted to the data by using nonlinear regression in Graphpad Prism 7 with residual errors weighted by the reciprocal of the square of the observed concentration.

Table 4.1: Patient characteristics and pharmacokinetic parameters for fentanyl in patients who received carbamazepine and in control patients.

	Mean (±SD) values	
	Control (n=6)	With Carbamazepine (n=11)
Patient Characteristics		
M/F	4/2	6/5
Race	6 White	10 White + 1 African American
Weight (kg)	72.6±12.2	80.1±24.23
Height (m)	1.70±0.09	1.72±0.12
BMI(kg/m^2)	24.74±2.66	27.26±8.06
Carbamazepine Dose (mg/day)	0	720
Serum Creatinine (mg/100mL)	0.87±0.11	0.77±0.20
Kinetic variables for fentanyl		
Volume of Distribution (L)	258±91	422±270
Volume of Distribution (L/kg)	3.60±1.15	5.45±3.13
Elimination half-life (hours)	3.28±0.89	3.09±1.340
Clearance (ml/min)	919±242*	1554±624*
Clearance (ml/min*kg)	13.21±4.83*	20.09±6.83*

*p≤0.05

Chapter 5: Pharmacokinetic analyses

Figure 5.1, 5.2, and 5.3 show mean concentrations of fentanyl at corresponding times in all subjects over 9 hours after fentanyl administration, along with the pharmacokinetic functions determined by nonlinear regression analysis. Figure 5.3 is a plot of data from Figure 5.1 and Figure 5.2.

Figure 5.2 shows mean maximum fentanyl plasma concentrations in controls were 13.7 ± 7.7 ng/mL. After entering the systemic circulation, the drug was distributed. The distribution phase lasting for approximately 15 minutes. After elimination equilibrium, the decline of plasma concentration was driven by elimination from the body. The elimination half-life was 197.1 ± 53.6 minutes and the area under the fentanyl plasma concentration-time curve ($AUC_{0-\infty}$) was 232.6 ± 70.0 ng*min/ml. The clearance of fentanyl is 13.2 ± 4.8 ml/min/kg.

Table 5.1, 5.2, and 4.1 show the coadministration of carbamazepine was associated with decreased fentanyl concentrations and lower $AUC_{0-\infty}$ to 150.3 ± 65.0 ng*min/ml ($P<0.05$, Figure 5.4) and increased systemic clearance of fentanyl to 20.1 ± 6.8 ml/min/kg. ($P<0.05$, Figure 5.4). Compared to controls, maximum plasma concentration of fentanyl was reduced to 6.9 ± 4.2 ng/mL.

Patients with obesity or burn injury can have altered volume of distribution [24,25]. To investigate whether alteration of fentanyl volume of distribution was influenced by the body weight or BMI, regression analysis was done, Figure 5.5 shows that body weight

and BMI accounted for an insignificant fraction of the overall variance in V_d and clearance.

Figure 5.1: Mean plasma fentanyl concentrations (±SE) following 200 µg fentanyl in patients with carbamazepine.

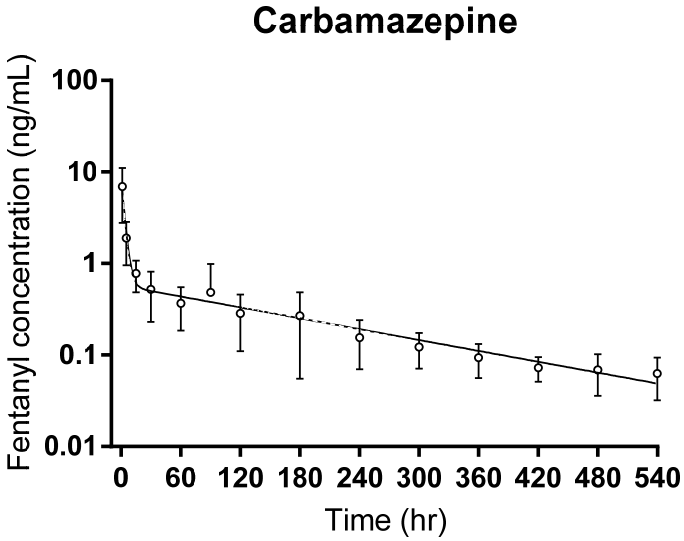


Figure 5.2: Mean plasma fentanyl concentrations (±SE) following 200 µg fentanyl in control patients.

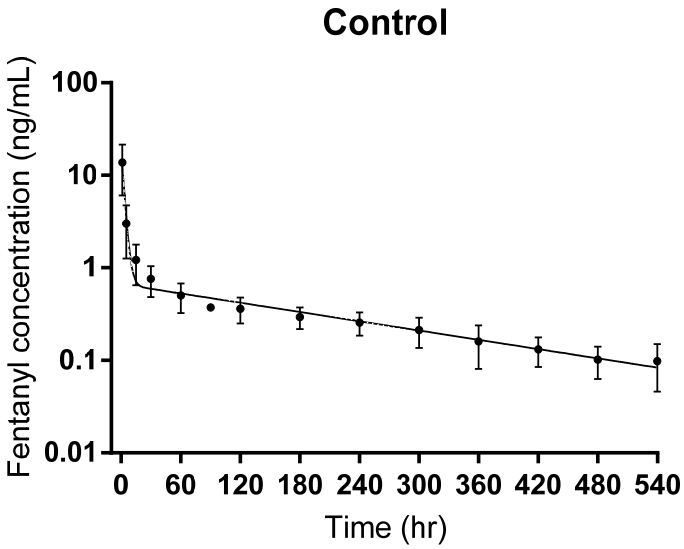


Figure 5.3: Mean plasma fentanyl concentrations (±SE) following 200 µg fentanyl in the two groups patients shown in Figure 4 and Figure 5. (Solid line is control group and dashed line is the carbamazepine group)

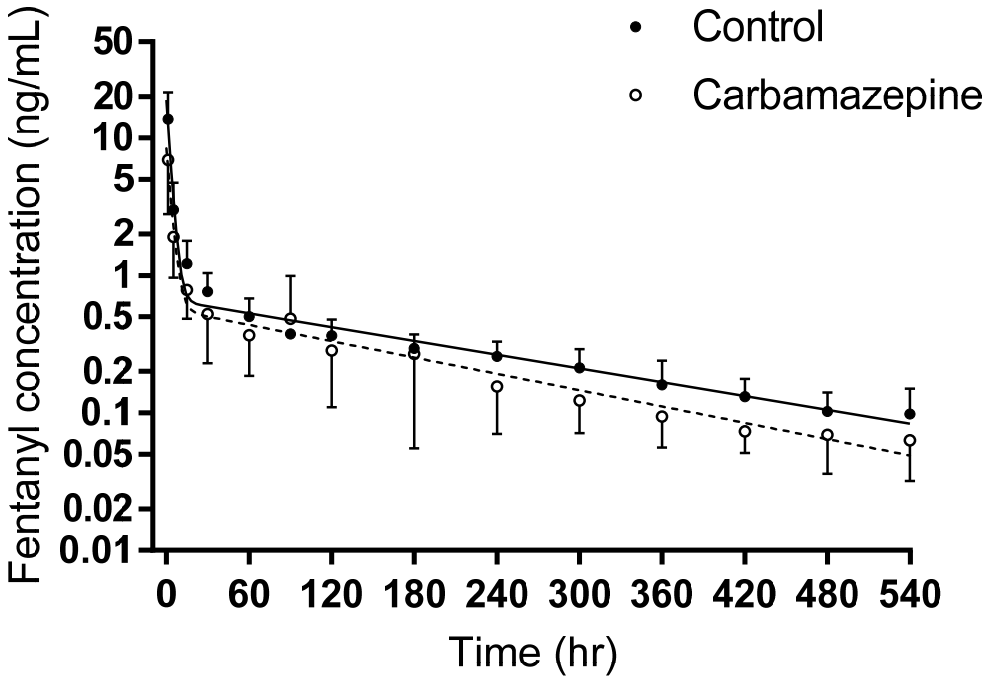


Figure 5.4: Statistical analysis of pharmacokinetic parameters. (*P<0.05)

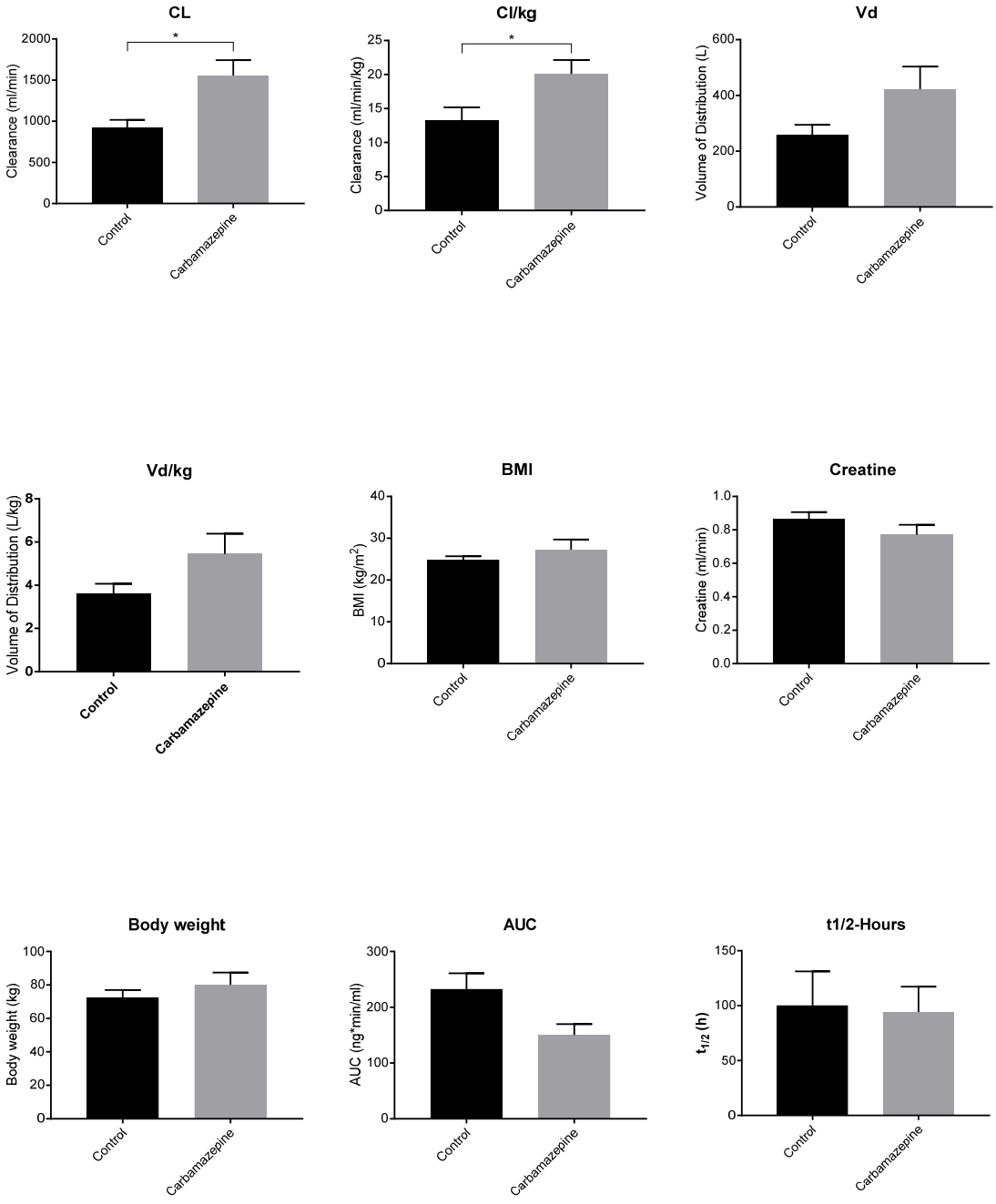


Figure 5.5: Relation of fentanyl plasma clearance and volume of distribution versus body weight and BMI.

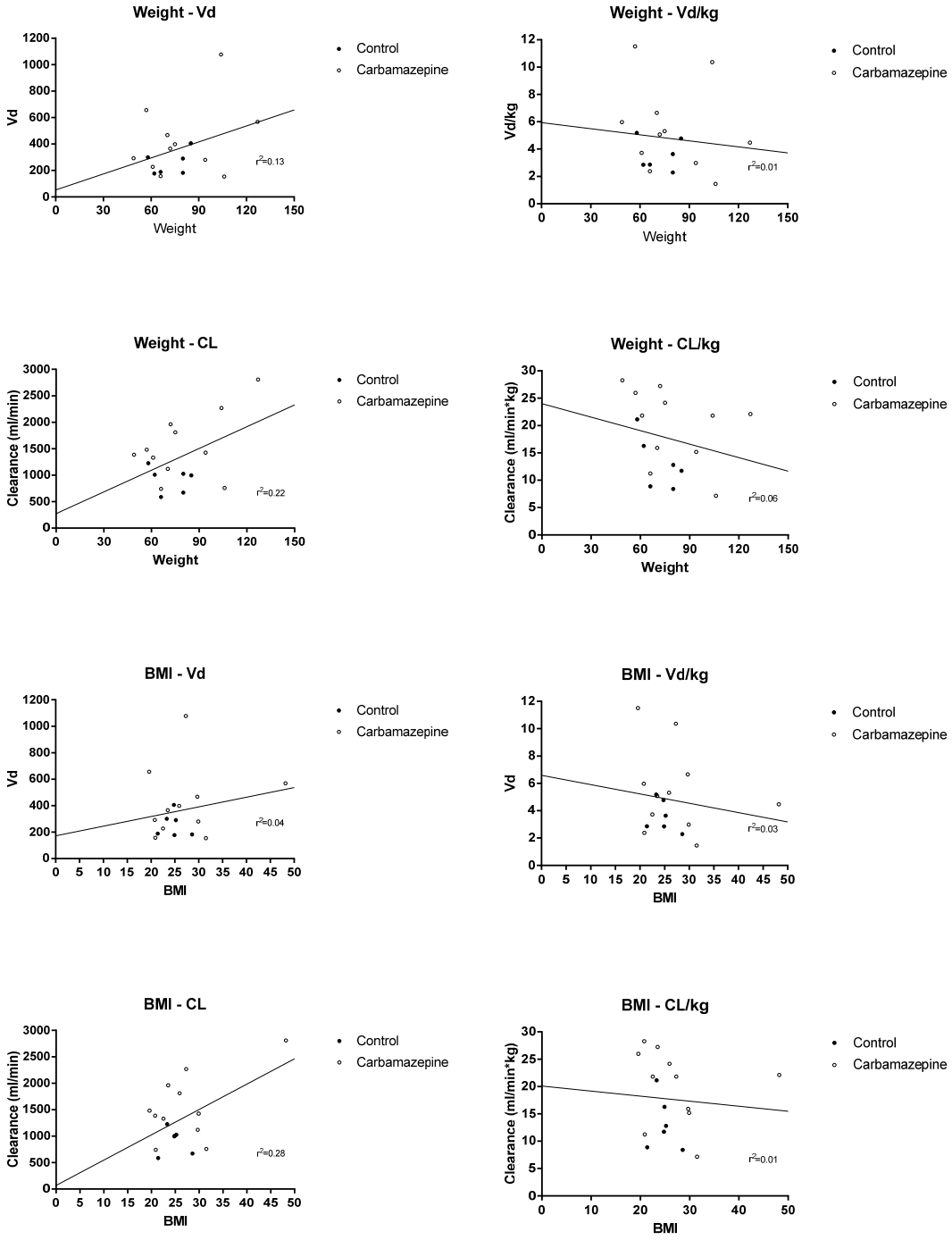


Table 5.1: The pharmacokinetic parameters in control patients.

Patient number	BMI (kg/m^2)	t1/2β (min)	t1/2β (h)	Vd (Liters)	Vd/kg (Liters/kg)	AUC (ng*min/ml)	Cl (ml/min)	Cl/kg (ml/min/kg)
1	28.1							
3	21.6							
4	24.8	281.88	4.70	405.54	4.77	200.56	997.22	11.73
5	21.4	223.60	3.73	188.98	2.86	341.39	585.84	8.88
6	24.9	121.67	2.03	177.15	2.86	198.18	1009.20	16.28
7	28.6	188.61	3.14	182.97	2.29	297.43	672.42	8.41
8	23.3	169.97	2.83	300.77	5.19	163.06	1226.53	21.15
18	25.2	196.58	3.28	290.76	3.63	195.08	1025.22	12.82
Mean	24.7	197.05	3.28	257.69	3.60	232.62	919.41	13.21
SD	2.7	53.64	0.89	91.21	1.16	69.99	241.62	4.83

(Patient 1and 3 had an insufficient duration of sampling to allow pharmacokinetic analysis)

Table 5.2: The pharmacokinetic parameters in patients receiving carbamazepine.

Patient number	BMI (kg/m^2)	Dose (μg) Carbamazepine	t1/2β (min)	t1/2β (h)	Vd (Liters)	Vd/kg (Liters/kg)	AUC (ng*min/ml)	Cl (ml/min)	Cl/kg (ml/min/kg)
2	29.7	200 bid	289.78	4.83	467.93	6.66	178.68	1119.30	15.92
9	23.5	400 bid	129.20	2.15	365.63	5.08	101.96	1961.63	27.24
10	27.3	200 tid	329.29	5.49	1077.40	10.36	88.19	2267.94	21.81
11	25.9	200 qid	152.41	2.54	398.46	5.31	110.36	1812.20	24.16
12	20.9	N/A	146.92	2.45	157.21	2.38	269.64	741.74	11.24
13	20.8	100 tid	146.51	2.44	292.93	5.98	144.31	1385.87	28.28
14	31.5	700 bid	141.23	2.35	154.70	1.46	263.42	759.25	7.16
14-2	19.6	400+400+200	306.97	5.12	656.26	11.51	134.97	1481.83	26.00
15	22.5	200 tid	118.41	1.97	227.30	3.73	150.31	1330.62	21.81
16	48.2	200+600	140.23	2.34	568.38	4.48	71.19	2809.53	22.12
17	29.9	K 400 bid, P 100	136.31	2.27	280.61	2.99	140.16	1426.92	15.18
Mean	27.3		185.20	3.09	422.44	5.45	150.29	1554.26	20.08
SD	8.1		80.32	1.34	269.78	3.13	65.01	624.40	6.83

Chapter 6: Discussion

The study was designed to define the pharmacokinetic component of the increased requirement of fentanyl caused by phenytoin or carbamazepine, commonly used antiepileptic drugs. Our results demonstrated that epileptic patients treated chronically with one of those anticonvulsants have higher clearance of fentanyl than controls. The half-life and volume of distribution were not significantly different between groups.

Clearance reflects the elimination of the drug from the body. This drug elimination generally results from liver metabolism or excretion by the kidneys. For every drug, each organ of elimination has its own clearance including renal clearance, hepatic clearance and others. For drugs such as fentanyl which is mainly metabolized by the liver, alterations in hepatic enzymatic activity, plasma protein binding, and liver blood flow, whether they be caused by a disease state, drug interaction, genetic or species reasons, may influence intrinsic drug clearance.

Carbamazepine stimulates the activity of hepatic drug metabolizing enzymes, including CYPs 2C9 and 3A4[26]. The primary route of fentanyl clearance is N-dealkylation to norfentanyl, and this process is predominately mediated by cytochrome P450 3A4 (CYP3A4) [27,28,29]. The increased requirement of fentanyl in epileptic patients might be the result of induction of CYP3A4 enzymes by carbamazepine. In addtion to their enzymatic inductive effect, carbamazepine can also induce the proliferation of the smooth endoplasmic reticulum in the liver cells, leading to hypertrophy of liver cells, which, together with hyperplasia of hepatocytes, can cause increased liver size and increased

hepatic blood flow in patients [30]. Since the enzymatic activity and liver blood flow both can be major determinants of hepatic clearance, for high clearance drugs such as fentanyl, the inductive effect of carbamazepine on fentanyl clearance may involve two mechanisms [31].

Another explanation for the increased fentanyl requirement may be alterations in the activity of P-glycoprotein efflux transporters. P-gp (P-glycoprotein 1), also known as multidrug resistance protein 1 (MDR1) or ATP-binding cassette sub-family B member 1 (ABCB1), is an important protein in the cell membrane that transports many foreign substances out of cells [32]. P-gp is extensively distributed and expressed in the intestinal epithelium as well as the blood brain barrier. Research has shown that carbamazepine is an inducer of the P-gp transporter, and fentanyl is a substrate of the P-gp transporter [32,33]. Since more fentanyl is transported back into the blood by the P-gp transporter, less fentanyl will cross the blood brain barrier and enter the central neural system, causing increased fentanyl requirements in the patients. A final possible mechanism for increased fentanyl requirement in these patients may involve alterations in the central nervous system sensitivity to effects of the opioids caused by the AEDs.

Chapter 7: Conclusion and future directions

Our results indicate that carbamazepine or phenytoin can significantly alter fentanyl clearance in humans. This effect is driven by either induction of hepatic metabolizing enzyme, or an increased hepatic blood flow, caused by carbamazepine or pheytoin, causing increased fentanyl requirements in the patients. In the future, fentanyl doses for patients with antieptieptic treatments need to be optimized to maintain its anesthetic and analgesic effect.

In our study, due to the limited number of patients, we do not perform a correlation study between dose of carbamazepine and increased clearance. To fully understand how carbamazepine increases fentanyl requirements in patients, we need to conduct a dose-dependence study of carbamazepine on the clearance of fentanyl.

To characterize other factors that might contribute to the increased fentanyl requirements in patients, we should investigate the effect of antiepileptic treatments on the activity of the P-gp transporter. We also need to define alterations in the central nervous system sensitivity to opioids by the AEDs.

Chapter 8: Bibliography

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