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Pharmacokinetics of a Combination of Δ^9-Tetrahydro-cannabinol and Celecoxib in a Porcine Model of Hemorrhagic Shock

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ABSTRACT: Hemorrhagic shock involves loss of a substantial portion of circulating blood volume leading to diminished cardiac output and oxygen delivery to peripheral tissues. In situations where an immediate resuscitation cannot be provided, pharmacotherapy with a novel combination of Δ^9-tetrahydro-cannabinol (THC) and celecoxib (CEL) is currently investigated as an alternative strategy to prevent organ damage. In the present study, 28 Yorkshire × Landrace pigs were used to study the pharmacokinetics of THC and CEL in an established porcine model of hemorrhagic shock. Pigs in hemorrhagic shock received 0.5, 1 or 4 mg/kg THC and 2 mg/kg CEL, while normotensive pigs received 1 mg/kg THC and 2 mg/kg CEL by intravenous injection. THC and CEL plasma concentrations were simultaneously determined by LC-MS/MS. Pharmacokinetic parameters and their between animal variability were obtained using standard non-compartmental analysis as well as a compartmental analysis using nonlinear mixed effects modeling. The concentration–time profiles of THC and CEL followed a multi-exponential decline and their pharmacokinetics were similar in hemorrhagic shock and normotensive conditions, despite the substantial change in hemodynamics in the animals with shock. This interesting finding might be due to the pharmacologic effect of the THC/CEL combination, which is intended to maintain adequate perfusion of vital organs in shock. Overall, this study established THC and CEL pharmacokinetics in a porcine shock model and provides the basis for dose selection in further studies of THC and CEL in this indication. Copyright © 2010 John Wiley & Sons, Ltd.

Key words: tetrahydro-cannabinol; celecoxib; hemorrhagic shock; pharmacokinetics

Introduction

Hemorrhagic shock involves the loss of a substantial portion of circulating blood volume leading to diminished cardiac output and oxygen delivery to the peripheral tissues. If untreated, such significant loss of intravascular volume may lead to hemodynamic instability, decreased tissue perfusion, cellular hypoxia, microcirculatory damage, multiple organ failure and ultimately death. Nearly 50% of all patients suffering from hypovolemia with hemorrhagic shock die within the first 24 h [1].

A well established therapeutic dogma has been to restore blood volume rapidly by resuscitation and achieve normal physiological parameters within the so-called 'golden hour', a time period allowed for medical personnel to reverse shock, in order to preserve organ function and to prevent death [2]. But in situations where an immediate resuscitation cannot be provided, an alternative strategy that can prevent the organ damage and increase the chances of survival until the patient is hospitalized becomes essential.

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Cannabinoids and their synthetic and endogenous analogs have long been known to affect a broad range of physiological functions, including cardiovascular variables. The endogenous cannabinoid system has been implicated recently in the mechanism of hypotension associated with hemorrhagic, endotoxic, as well as cardiogenic shock [3]. Δ^9-Tetrahydro-cannabinol (THC) which is the main phytocannabinoid present in *Cannabis sativa* L. is responsible for the pharmacological effects of the plant. THC was found to be beneficial for survival in shock in murine models via activation of peripheral CB_1 cannabinoid receptors [4].

Cyclo-oxygenase (COX)-2 catalyses the conversion of arachidonic acid to prostaglandin H2, the precursor for prostaglandins and thromboxane A2, triggering vasodilation and platelet inhibition. Celecoxib (CEL), a selective COX-2 inhibitor, prevents vasodilation by inhibiting this conversion. This effect of COX-2 inhibition in combination with THC is hypothesized to increase the chances of survival in hemorrhagic shock. Several authors reported THC pharmacokinetics in different species under normotensive conditions, but this is the first study in which THC pharmacokinetics, in combination with CEL, is explored in a porcine model of hemorrhagic shock.

The objective of the present study was to evaluate the pharmacokinetics of a novel binary drug combination consisting of THC and CEL for the treatment of hemorrhagic shock in an established animal model for this condition. The results of this study are intended to provide a rational basis for dose selection of the binary drug combination used in this study for further preclinical and clinical development.

Methods

Study design

The pharmacokinetics (PK) of THC and CEL in hemorrhagic shock were studied by intravenously administering a formulation containing both drugs to pigs with hemorrhagic shock as well as normotensive pigs. This study consisted of four different groups based on the dose each animal received in either the normotensive or shock state. Three different doses of THC (0.5 mg/kg, 1 mg/kg and 4 mg/kg) each in combination with 2 mg/kg CEL were administered to pigs with shock, while 1 mg/kg THC along with 2 mg/kg CEL was administered to normotensive pigs. This study setup is outlined in Table 1.

Table 1. Study groups based on the dose of THC and CEL each animal received in a given condition

Group	THC (mg/kg)	CEL (mg/kg)	Condition	No. of pigs
1	1	2	Normotensive	6
2	0.5	2	Shock	6
3	1	2	Shock	10
4	4	2	Shock	6

Animals, drug administration and blood sampling

This research adhered to the 'Principles of Laboratory Animal Care' and the 'Guide for Care and Use of Laboratory Animals'. The study protocol was approved by the Institutional Animal Care and Use Committee of The University of Tennessee Health Science Center. The experiments were performed in 28 healthy female Yorkshire X Landrace pigs aged 6–8 months and weighing 49.1 ± 2.6 kg that were purchased from Oak Hill Genetics (Ewing, IL). They were fed with a standard pig diet and allowed free access to drinking water. The hemorrhagic shock model used in this study was pressure controlled, wherein the mean arterial pressure was decreased to 35–40 mmHg, representing a mean blood loss of approximately 35% [5]. Animals were held in shock for 30 min prior to drug administration.

The THC and CEL concentrations obtained from three of the animals from the 4 mg/kg THC and 2 mg/kg CEL-shock group were excluded from further analysis because these animals had a very short survival time due to cardiac failure, resulting in erratic concentration–time profiles confounded by the related dramatic changes in hemodynamics.

Based on pilot studies, it was determined that the total experimental time post-injection would be 8 h. Hence blood specimens were collected between 0 and 8 h at 5, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420 and 480 min. Blood

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samples of 1 ml were drawn from a femoral artery catheter at the designated intervals and placed into K3-EDTA anti-coagulated sampling tubes. These blood samples were centrifuged at $2000 \times g$ for 10 min at 4°C after collection to separate the cellular blood components from the plasma. The collected plasma was transferred to clean vials and stored at −80°C until analysis. Since THC and CEL are both not preferentially distributed into red blood cells [6,7], plasma concentrations were used for the PK evaluation in this study.

Bioanalytical assay for THC and CEL

Sample preparation. A simple protein precipitation method using acetonitrile was employed for sample preparation. Structurally similar analogs KMII33 (6,6,9-trimethyl-3-(1-methyl-1-phenylethyl)-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-1-ol) and nimesulide were used as internal standards for THC and CEL. 50 µl of plasma sample was transferred into a 1.5 ml tube and protein was precipitated using 200 µl cold acetonitrile containing both internal standards at a concentration of 1 µg/ml. The mixture was vortexed for 1 min and kept on ice for 20 min. The samples were then centrifuged at $3000 \times g$ for 10 min at 4°C. The clear supernatant was diluted if necessary, and subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) for analysis.

Assay procedure and instrumentation. A rapid and sensitive LC-MS/MS method was developed for simultaneous determination of THC and CEL along with their internal standards KMII33 and nimesulide. The LC-MS/MS system consisted of an Applied Biosystems/MDS Sciex API 3000 triple quadrupole mass spectrometer that was operated in negative ion mode with electrospray ionization using a Turbo Ion Spray interface and linear accelerator (LINAC) collision cell. Chromatographic separation of the analytes was performed using a C8 column (50 mm × 2.1 i.d., 3.5 µm) with guard column, applying gradient elution with 5 mM ammonium acetate (pH 6.8) (A) and acetonitrile (B). The flow-rate was 0.3 ml/min and the gradient was as follows: 0–1.01 min; 30% B; 1.01–3.01 min, linear from 30% to 90% B; 3.01–6.01 min, 90% B; 6.01–7.0 min, linear from 90% to 30% B; 7–10 min, 30% B.

Optimization of the mass analyser parameters for LC-MS/MS data acquisition on the API 3000 system was achieved by constant infusion of 10 µl/min analyte solutions (1 µg/ml) by a syringe pump coupled to the mass spectrometer. The final conditions of the LC-MS/MS method were set to turbo ionspray gas 7 l/min, nebulizer gas 12 psi, curtain gas 12 psi, collision gas 8 psi, ionspray voltage −4500 V, temperature 400°C.

Characteristic m/z ratios for precursor/product ion transfers as listed in Table 2 were used in the mass analyser's multiple reaction monitoring mode to quantify drug concentrations. Concentrations for each analyte were determined based on the ratio of its peak area to the peak area of the internal standard. A calibration curve with six calibration standards (31.3, 62.5, 125, 250, 500 and 1000 ng/ml) covering the entire expected concentration range was used to relate peak area ratios of unknown samples to analyte concentrations.

Calibration and assay performance

Stock solutions of THC and CEL and a combined stock solution of both drugs were prepared in acetonitrile. Similarly, a combined stock solution using internal standards was prepared in acetonitrile. Calibration standards in blank pig plasma were prepared by spiking combined drug stock solution, covering a concentration range of

Table 2. Analytes and their mass spectrometric detection parameters

Analyte	Precursor ion	Product ion	Declustering potential	Focusing potential	Collision energy	Cell exit potential
THC	313	245.2	−40	−100	−35	−20
KMII33[a]	361.2	293.1	−60	−100	−40	−20
CEL	380	316.1	−40	−100	−30	−20
NIM[a]	307	229	−10	−100	−20	−20

[a]THC internal standard: KMII33; CEL internal standard: nimesulide.

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31.3 ng/ml to 1000 ng/ml. The prepared stock solutions were stable throughout the study when stored at 4°C. Quality control samples showed stability in plasma for 3 months when stored at −80°C. They were also stable after three freeze–thaw cycles.

The bioanalytical method for both THC and CEL was linear over a range of 31.3 to 1000 ng/ml with correlation coefficients of $r > 0.997$. The lower limit of quantification, defined as the lowest concentration on the calibration curve with the signal-to noise ratio >10, was found to be 31.3 ng/ml for each analyte. The mean accuracy ranged from 94.5% to 106.0% and the intra-day and inter-day precision was between 3.2–9.7% and 5.1–11.5%, respectively [8].

Quality control of formulations

The THC and CEL combined drug formulations were prepared in the pharmaceutics laboratory at the University of Tennessee Health Science Center, Memphis, TN. They were freshly prepared in a total volume of 70 ml consisting of a solvent mixture of Cremophor: ethanol: saline in %v/v ratio of 4: 4: 92. The actual concentrations of THC and CEL in the dosage form were determined using the LC-MS/MS method for simultaneous estimation of THC and CEL as described above.

Pharmacokinetic analysis

A pharmacokinetic analysis based on the measured plasma concentrations for THC and CEL was performed using standard non-compartmental methods as well as a compartmental nonlinear mixed effects modeling approach.

Non-compartmental analysis

For non-compartmental analysis (NCA), the terminal half-life ($t_{1/2}$) was obtained from the relationship $0.693/\lambda_z$ where λ_z is the negative slope of the terminal linear phase of a plot of the natural logarithm of plasma concentration (C) versus time (t). λ_z was estimated by linear regression analysis of those data points that appeared by visual inspection to be in the apparent terminal linear phase. The area under the plasma concentration–time curve (AUC) and the area under the first moment curve ($AUMC$) were calculated using the log-linear trapezoidal rule. For the calculation of the terminal portion of $AUC_{0\text{-}\infty}$ beyond the last measured data point the factor C_i/λ_z was added, and for $AUMC_{0\text{-}\infty}$ the factor $C_i \cdot t/\lambda_z + (C_i/\lambda_z)^2$ was added, where C_i is the last measured concentration at time t_i. Clearance (CL) was calculated as dose (D) divided by $AUC_{0\text{-}\infty}$. The volume of distribution at steady state (V_{ss}) was calculated as $D \cdot AUMC_{0\text{-}\infty}/(AUC_{0\text{-}\infty})^2$. C_0, the initial concentration at time $t = 0$, was back-extrapolated from the first two measured concentration values based on log-linear regression. The volume of distribution at pseudo-distribution equilibrium ($V_{\lambda z}$) was calculated as $D/(\lambda_z \cdot AUC_{0\text{-}\infty})$. Dose-normalized exposure ($AUC_{0\text{-}\infty}/D$) was calculated to assess dose-proportionality among the administered THC dose levels. One-way analysis of variance (ANOVA) with posthoc comparisons using Bonferroni correction was performed to compare group means of parameters for the different treatments.

Compartmental pharmacokinetic analysis

A compartmental pharmacokinetic analysis was performed by nonlinear mixed effects modeling using a NMQual (Metrum Institute, Augusta, ME) installation of the NONMEM program (Version VI, Level 2.0, Icon Development Solutions, Ellicott City, MD). As the structural model component, one, two and three compartment pharmacokinetic models with linear disposition were evaluated. As the statistical model component, an exponential model was used to describe the between subject variability of pharmacokinetic parameters and a proportional, additive and a combined proportional/additive model were tested to describe the residual variability. The first-order conditional estimation method with η–ε interaction was employed for all runs. The appropriate structural model was chosen based on the examination of goodness-of-fit plots, changes in the objective function value (OFV) computed by NONMEM as well as the Akaike information criterion (AIC), calculated as $-2\log(\text{maximum likelihood}) + 2 \cdot k$ (k = the number of independently adjusted parameters within the model) [9,10]. For the THC analysis, condition (normotensive or shock) was

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evaluated as a covariate on THC clearance and volumes of distribution. For the CEL analysis, condition as well as the different dose levels of THC (0.5 mg/kg, 1 mg/kg and 4 mg/kg) were evaluated as categorical covariates on CEL clearance and volumes of distribution. The *AUC* of THC was evaluated as a continuous covariate for CEL clearance. A stratified approach to the univariate analysis was taken during the forward addition step of the covariate modeling, in that the condition was first evaluated and included in the model. Once the effect of condition on CEL pharmacokinetics had been accounted for, the different dose levels of THC or *AUC* of THC were evaluated in the model. A likelihood ratio test was employed to test the statistical significance of chosen covariates in hierarchical models. A decrease in OFV greater than 6.6 was considered to be statistically significant ($p = 0.01$).

Results and Discussion

The current study investigated the pharmacokinetics of a novel binary drug combination consisting of THC and CEL for the treatment of hemorrhagic shock in an established animal model for this condition. The pharmacokinetic evaluation comprised a traditional non-compartmental analysis as well as a nonlinear mixed-effects based compartmental approach. The population-based nonlinear mixed effects modeling approach is used increasingly in preclinical pharmacokinetic studies as it provides a better estimation of between study animal variability in pharmacokinetic parameters. In addition, applying simulation techniques, information from the derived population pharmacokinetic models can be extrapolated to explore future study designs. Thus, the objective of the current study was not only to establish the PK of THC and CEL in the shock model, but also to develop a population pharmacokinetic model to facilitate simulation studies for future dose selection in preclinical and clinical studies.

Quality control of formulations

In the formulations tested, the %mean (%CV) of nominal dose for THC and CEL was 98% (8.9%) and 89% (5.7%), respectively. This showed that the THC amounts in the formulations were close to the nominal dose strength while for CEL they were about 10% less indicating that the actual CEL dose given to pigs might be slightly lower than the nominal dose of 2 mg/kg.

THC pharmacokinetics

Figure 1 illustrates the mean concentration–time profiles of THC in different study groups following simultaneous intravenous administration of THC and CEL. The profiles of THC appear to follow a bi-exponential decline with concentrations for THC monitored up to 8 h. The initial distribution phase after intravenous administration seems to be largely completed after 1.0–1.5 h. This is consistent with various studies involving cannabinoids in humans, for which the plasma concentration–time profiles have been described with two [11], three [12–14] or even four [15] compartment models.

A dose-dependent increase in exposure with a higher C_0 and increased *AUC* can be observed as dose increases from 0.5 to 4 mg/kg in animals with shock. Furthermore, the average concentration–time profiles of 1 mg/kg THC in shock and normotensive pigs overlapped each other indicating similar pharmacokinetic behavior regardless of the shock status of the animals.

Table 3 summarizes the mean (%CV) for various THC pharmacokinetic parameters in different study groups from the NCA. THC seems to follow linear pharmacokinetics in the

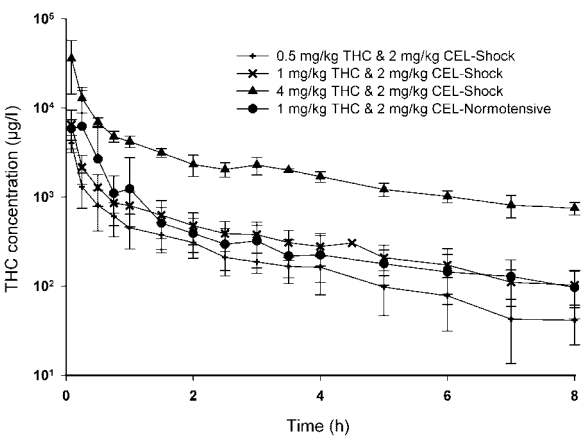

Figure 1. THC mean (±SD) concentration–time profiles for different doses of THC in shocked and normotensive pigs

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animals with shock, as indicated by a proportional increase in *AUC* with increase in dose with mean (%CV) values of 2758 (23.5%), 4798 (36.3%) and 25524 (15.3%) $\mu g \cdot h \cdot l^{-1}$ for 0.5, 1 and 4 mg/kg THC, respectively. This notion is supported by the fairly constant dose-normalized *AUC* values for THC in the three dose levels of animals with shock, ranging from 0.101 to 0.129 $h \cdot l^{-1}$. Comparing THC pharmacokinetics in normotensive and shock pigs, the terminal half-life of 2.08 h (18.3%) was significantly shorter ($p = 0.00045$) in normotensive animals than in animals with shock with average half-lives of 2.67 h (10.5%) to 2.91 h (15.9%). However, this observation is neither supported by clear differences in volume of distribution nor clearance between normotensive and shock animals. THC mean (%CV) *AUC* in normotensive pigs was 5436 $\mu g \cdot h \cdot l^{-1}$ (79.7%) and not significantly different from the *AUC* in shock animals at the same dose level, 4798 $\mu g \cdot h \cdot l^{-1}$ (36.3%). Overall, a relatively modest between-animal variability was observed among the listed parameters in shock pigs. Systemic exposure quantified as *AUC*, for example, ranged from coefficients of variation of 15.3% to 36.3%. In normotensive pigs, this variability seemed to be higher with *AUC* %CV values of 79.7%.

The pharmacokinetic parameters of THC from the compartmental analysis are summarized in Table 4. A three compartmental PK model (ADVAN11, TRANS4) was found to best describe the THC data on the basis of AIC in comparison with one and two compartmental models and hence was chosen as the structural base model. The goodness-of-fit of this pharmacokinetic model was assessed through the correlations between the observed and predicted individual and population THC plasma concentrations versus time and through the plots of the weighted residuals versus predicted concentrations and time (not shown). In the final model, an

Table 3. Mean and %CV for PK parameters for THC in different study groups derived by NCA

Group Parameter	Units	Normo 1 ($n = 6$)		Shock 0.5 ($n = 6$)		Shock 1 ($n = 10$)		Shock 4 ($n = 3$)	
		Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
Dose	mg	48.9	4.91	25.7	3.82	48.0	4.90	197	6.93
C_o	$\mu g \cdot l^{-1}$	8083	16.2	8755	76.7	11357	54.9	61036	72.6
$AUC_{0-\infty}$	$\mu g \cdot h \cdot l^{-1}$	5436	79.7	2758	23.5	4798	36.3	26965	12.0
$AUC_{0-\infty}/D$	$h \cdot l^{-1}$	0.112	79.7	0.107	22.3	0.101	38.2	0.137	13.5
V_{ss}	l	22.9	46.4	20.5	22.8	30.5	33.4	22.5	26.5
$V_{\lambda z}$	l	37.3	51.2	36.8	15.6	47.8	40.6	30.7	10.5
CL	$l \cdot h^{-1}$	12.6	47.8	9.75	23.7	11.2	36.4	7.40	14.8
$T_{1/2}$	h	2.08	18.3	2.67[a]	10.5	2.93[a]	9.71	2.91[a]	15.9

Normo 1, normotensive (1 mg/kg THC and 2 mg/kg CEL); Shock 0.5, shock (0.5 mg/kg THC and 2 mg/kg CEL); Shock 1, shock (1 mg/kg THC and 2 mg/kg CEL); Shock 4, shock (4 mg/kg THC and 2 mg/kg CEL).
[a]$p < 0.05$ compared with normotensive animals.

Table 4. PK parameters of THC obtained by the compartmental analysis using nonlinear mixed effects modeling

Parameter		Estimate	%RSE
CL (l/h)	Clearance	10.3	7.14
V1 (l)	Central compartment volume	5.62	8.02
V2 (l)	Peripheral compartment 1 volume	19.0	9.11
V3 (l)	Peripheral compartment 2 volume	7.69	10.9
Q2 (l/h)	Inter-compartmental clearance (central and peripheral compartment 1)	6.98	18.1
Q3 (l/h)	Inter-compartmental clearance (central and peripheral compartment 2)	13.7	7.00
BSV (variance and %CV)	Between-subject variability		
BSV-CL		0.113 (33.6)	24.4
BSV-V1		0.0791 (28.1)	40.5
BSV-V2		0.0901 (30.0)	29.1
BSV-Q2		0.307 (55.4)	33.6
RV (%CV)	Residual variability		
Proportional error		12.2	16.0

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exponential error structure for BSV was used to describe CL, V1 (volume of the central compartment), V2 (volume of peripheral compartment 1) and Q2 (inter-compartmental clearance between central and peripheral compartment 1), and a proportional error model was used to describe residual variability. The shock/normotensive condition used as a covariate on THC clearance did not yield a significant difference in the OFV and hence it was not used in the final model. This further supports the results from the noncompartmental analysis that the shock/normotensive condition does not significantly impact the clearance or the volume of distribution of THC.

In humans, THC is a high hepatic extraction drug, with metabolism mainly mediated by the cytochrome P450 (CYP) 2C family of drug metabolizing enzymes in the liver [16–20]. For the pig breed used in this study, the hepatic blood flow has been reported as 93 l/h [21,22], and the observed THC clearance was 12.6 l/h. Thus, THC can be considered a low hepatic extraction drug in swine for which hepatic clearance is largely determined by hepatic enzyme activity rather than hepatic blood flow. In the untreated hemorrhagic shock in the animal model used in this study, liver blood flow was reported to be reduced to 23% of its normal value [22], i.e. approximately 21 l/h, a value still substantially higher than the observed average THC clearance range of 7.40–11.2 l/h. This observation may explain the lack of a major effect of hemorrhagic shock on the clearance of THC.

A further contributing factor may be the pharmacological activity of THC. Previously it was shown that activation of cannabinoid CB_1 receptors is beneficial for survival in shock [4]. Although the underlying mechanism is unclear, a favorable redistribution of cardiac output or improved microcirculation by localized vasodilatation has been described [23]. In such conditions the microvasculature in peripheral tissue beds may undergo selective constriction in response to a CB_1 agonist, shunting the cardiac output as well as the blood flow to vital organs in order to prevent their failure. Overall, THC pharmacokinetics in shock pigs is predictable and similar to the disposition characteristics observed for THC in normotensive pigs.

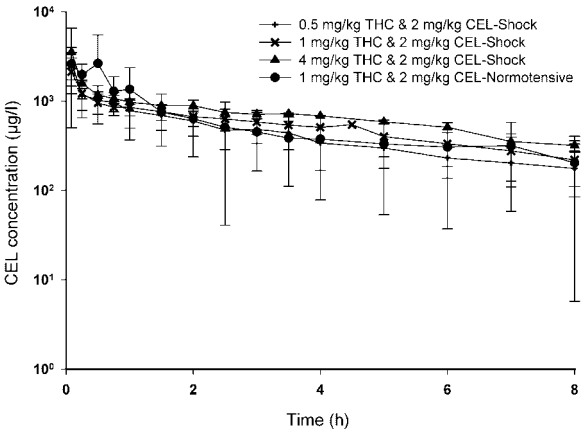

Figure 2. Celecoxib mean (±SD) concentration–time profiles for different doses of CEL in shocked and normotensive pigs

CEL pharmacokinetics

The plasma concentration–time profiles of CEL appear to follow a bi-exponential decline as shown in Figure 2. Similar to THC, CEL exhibits moderate between-animal variability in the investigated pharmacokinetic parameters derived by NCA. Table 5 summarizes the mean (%CV) for various parameters in the different study groups for CEL. From the NCA, CEL systemic exposure is not different between shock and normotensive pigs. The mean *AUC* (%CV) in shock was 5801 (29.5%) $\mu g \cdot h \cdot l^{-1}$ while that in normotensive pigs was 6114 (64.5%) $\mu g \cdot h \cdot l^{-1}$. However, there seems to be an increasing *AUC* secondary to a decreasing clearance of CEL with increasing doses of THC, although this trend did not reach statistical significance, probably due to the limited number of animals studied. Mean clearance was $22.9 l \cdot h^{-1}$ (26%) in the 0.5 mg/kg THC dose group, $18.2 l \cdot h^{-1}$ (35.8%) in the 1 mg/kg THC dose group, and $11.5 l \cdot h^{-1}$ (5.8%) in the 4 mg/kg THC dose group. The resulting average half-lives increased with dose from 2.92 h (34.4%) to 3.59 h (29.8%) and 4.78 h (24.0%).

The compartmental pharmacokinetic parameters of CEL are listed in Table 6. On the basis of AIC in comparison with a one compartmental model, a two compartmental model was found to best describe the CEL data and therefore was chosen as the structural base

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Table 5. Mean and %CV for PK parameters for CEL in different study groups derived by NCA

Group Parameter	Units	Normo 1 ($n = 6$)		Shock 0.5 ($n = 6$)		Shock 1 ($n = 10$)		Shock 4 ($n = 3$)	
		Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
Dose	mg	97.8	4.92	103	3.84	96.0	4.92	98.6	6.90
C_0	$\mu g \cdot l^{-1}$	3020	37.6	5794	109	2943	56.0	5655	108
$AUC_{0-\infty}$	$\mu g \cdot h \cdot l^{-1}$	6114	64.5	4723	25.3	5801	29.5	8584	5.60
V_{ss}	l	68.4	40.6	88.3	27.0	86.6	32.0	74.2	27.4
$V_{\lambda z}$	l	88.7	36.6	95.2	38.5	89.5	35.7	78.6	20.0
CL	$l \cdot h^{-1}$	23.5	62.7	22.9	26.0	18.2	35.8	11.5	5.81
$T_{1/2}$	h	3.44	65.0	2.92	34.4	3.59	29.8	4.78	24.0

Normo 1, normotensive (1 mg/kg THC and 2 mg/kg CEL); Shock 0.5, shock (0.5 mg/kg THC and 2 mg/kg CEL); Shock 1, shock (1 mg/kg THC and 2 mg/kg CEL); Shock 4, shock (4 mg/kg THC and 2 mg/kg CEL).

Table 6. PK parameters of CEL derived by compartmental analysis using nonlinear mixed effects modeling

Parameter		Estimate	%RSE
CL (l/h)	Clearance	17.9	10.3
V1 (l)	Central compartment volume	41.0	15.5
V2 (l)	Peripheral compartment volume	37.8	12.6
Q	Inter-compartmental clearance (central and peripheral compartment)	67.3	38.6
BSV (variance and %CV)	Between-subject variability		
BSV-CL		0.215 (46.4)	23.7
BSV-V1		0.152 (39.0)	34.9
BSV-V2		0.101 (31.6)	49.7
RV (%CV)	Residual variability		
Proportional error		11.3	17.3

model. In the final model, an exponential error model for BSV was used to describe CL, V1 (volume of the central compartment) and V2 (volume of peripheral compartment), and a proportional residual error model was used to describe residual variability. Since we suspected a dose dependent decrease of CEL clearance with increasing doses of THC from the NCA, in addition to condition (normotensive/shock), dose level of THC and *AUC* of THC were also used as covariates to study the clearance among different study groups. However, owing to the small number of animals in some of the dose groups, there was no significant difference in the OFV when dose or *AUC* of THC were used as a covariate. Condition as a covariate also did not significantly decrease the OFV as per the likelihood ratio test. The observed and model-predicted individual concentration–time profiles for selected animals from different dose and treatment groups overlaid with predicted population mean profiles for both THC and CEL are shown in Figure 3.

The comparison of hepatic blood flow ($\sim$931/h) and the observed CEL clearance (23.51/h) in normotensive animals indicates that CEL, similar to THC, is a low hepatic extraction drug in swine. A potential decrease in CEL clearance with increasing THC dose suggests a drug–drug interaction between both drugs. Although there are no reports so far that describe a clinical interaction among these drugs, potential mechanisms for the observed phenomenon could be changes in plasma protein binding of CEL or in the intrinsic hepatic clearance of CEL. The major elimination pathway for CEL in humans is hepatic metabolism by the CYP2C9 enzyme [24]. As THC is also primarily metabolized through the CYP2C family [25], competitive inhibition of CEL metabolism by THC might be a potential explanation for the observed interaction.

Conclusion

Δ^9-Tetrahydro-cannabinol and celecoxib exhibit predictable and reproducible pharmacokinetics in a pig model of hemorrhagic shock. The pharmacokinetics of THC and CEL were similar in hemorrhagic shock and normotensive conditions.

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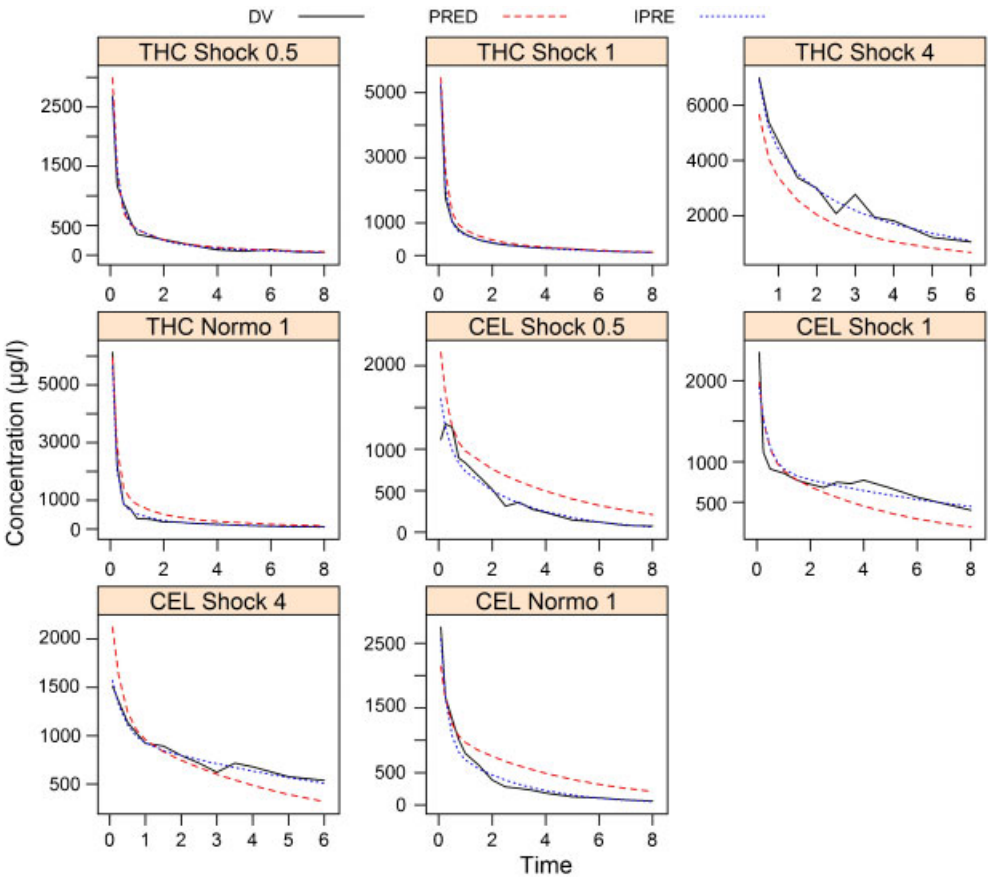

Figure 3. Observed (DV) and model predicted (IPRE) individual concentration–time profiles for selected animals from different dose and treatment groups for THC and CEL overlaid with predicted population mean (PRED) profiles

This interesting finding might be at least partially due to the pharmacological effect of the THC/CEL combination, which is intended to maintain adequate liver perfusion in shock, supporting the hypothesis that there is selective constriction of microvasculature in peripheral tissues in response to a CB_1 agonist, shunting cardiac output and blood flow to vital organs in order to prevent their failure. By establishing THC and CEL pharmacokinetics in pigs, this study provides the basis for dose selection in further preclinical and clinical studies to evaluate the efficacy and safety of the THC/CEL binary drug combination for the treatment of hemorrhagic shock.

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