Semi-Mechanistic Modeling of the Effects of Blast Overpressure Exposure on Cefazolin Pharmacokinetics in Mice


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RUNNING TITLE PAGE: Modeling Blast Effects of Cefazolin PK in Mice

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Number of Text Pages: 27
Number of Figures: 9 (5 in Supplemental Material)
Number of Tables: 5 (3 in Supplemental Material)
Number of References: 26
Number of Words, Abstract: 189
Number of Words, Introduction: 608
Number of Words, Discussion: 1239

Nonstandard Abbreviations:
AUC (Area under the Curve)
DILI (Drug-Induced Liver Injury)
LLOQ (Lower Limit of Quantification)
NCA (Non-Compartmental Analysis)
OEF (Operation Enduring Freedom)
OIF (Operational Iraqi Freedom)
PK (Pharmacokinetic)

Recommended Section: Chemotherapy, Antibiotics and Gene Therapy
ABSTRACT

Cefazolin is a first-line antibiotic to treat infection related to deployment-associated blast injuries. Prior literature demonstrated a 331% increase cefazolin liver area under the curve (AUC) in mice exposed to a survivable blast compared to controls. We repeated the experiment, validated the findings and establishing a semi-mechanistic two-compartment pharmacokinetic (PK) model with effect compartments representing the liver and skin. We found that blast statistically significantly increased the pseudo-partition coefficient to the liver by 326% (95% confidence interval: 76 – 737%), which corresponds to the observed 331% increase in cefazolin liver AUC described previously. To a lesser extent, plasma AUC in blasted mice increased 14 - 45% compared to controls. Nevertheless, the effects of blast on cefazolin PK were transient, normalizing by 10 hours post-dose. It is unclear as to how this blast effect temporally translates to humans; however, given the short-lived effect on PK, there is insufficient evidence to recommend cefazolin dosing changes based on blast overpressure injury alone. Clinicians should be aware that cefazolin may cause drug-induced liver injury with a single dose and the risk may be higher in patients with blast overpressure injury based on our findings.
SIGNIFICANCE STATEMENT

Blast exposure significantly, but transiently, alters cefazolin pharmacokinetics in mice. The questions of whether other medications or potential long-term consequences in humans need further exploration.
INTRODUCTION

During Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF), over 52,000 US Service members were wounded in action (Weintrob et al., 2018). Over three-fourths of casualties of OEF/OIF (Belmont et al., 2010) and two-thirds of deaths in OIF were due to explosive injuries (Wolf et al., 2009). More than one-third of casualties evacuated to military hospitals acquired an infection with more than half being skin, soft tissue, or bone infections (Weintrob et al., 2018). Despite the high severity of injuries, particularly from improvised explosive devices, most casualties with traumatic amputations arriving alive at a Role II or III military treatment facility will survive to transfer to higher levels of care (Godfrey et al., 2017). Traumatic amputations, particularly in OEF, were almost always caused by improvised explosive device blasts and the risk of infection rose with the number of limbs lost (Godfrey et al., 2017) and with increasing severity of injury (Stewart et al., 2019). Nearly one-half of traumatic or early surgical amputations developed an infection, including 68% with above knee amputations (Stewart et al., 2019). Early surgical wound debridement and antibiotic therapy are protective against combat-related extremity wound infections (Stewart et al., 2019). Cefazolin is the preferred antibiotic to prevent skin, soft tissue, and open fracture infections (Saeed et al., 2018). However, it is unknown how the pharmacokinetics of antibiotics in humans may be altered due to physiologic changes from blast injuries.

In a previous experiment, blast exposure resulted in significant pharmacokinetic differences of IV cefazolin in mice (Antonic et al., 2020). Following a survivable blast, liver area under the curve (AUC) increased over 4-fold and plasma half-life increased approximately 1.5-fold compared to control mice. Cefazolin is predominantly excreted unchanged in the urine, but the changes occurred in the absence of histological and biochemical evidence of renal injury. Similarly, liver accumulation occurred without histological evidence of gross liver injury and there was only mild, transient elevation of serum alanine transaminase. Serum albumin levels were unchanged between blasted and control mice.
One hypothesis explaining the pharmacokinetic changes may be due to increased vascular or tissue permeability (Antonic et al., 2020). This is supported by a rabbit blast model demonstrating microvessel injury in the lungs, liver, and kidneys causing time-dependent hemoconcentration and increased vascular permeability as measured by leakage of radiolabeled albumin (Zhang et al., 2011). Increased tissue binding was also a consideration; however, cefazolin is 80 – 90% protein bound and there was no evidence of change in serum albumin, making this explanation less likely (Vella-Brincat et al., 2007).

An additional hypothesis not previously explored is that changes in regional organ perfusion secondary to the blast may alter the delivery of cefazolin to specific organs. The initial phase of blast overpressure injury is a vasovagal reflex that decreases cardiac output, followed by a compensatory sympathetic activation with splanchnic vasoconstriction increasing portal perfusion to the liver and peripheral vasoconstriction decreasing perfusion to other end-organs (Cernak, 2015). Therefore, if cefazolin delivery to tissue is in part a function of organ perfusion, these regional changes in blood flow could explain increased cefazolin exposure to the liver and to a lesser extent to the central circulation, with a decrease in skin exposure.

Given the large variability in mouse pharmacokinetic (PK) studies, where due to ethical considerations each mouse is able to provide only a single plasma sample, we sought to repeat the experiment performed by Antonic et al. Our intent was to confirm the findings and perform an updated analysis utilizing semi-mechanistic pharmacokinetic modeling to provide further support for the hypothesis described above. In addition, we analyzed cefazolin concentrations in skin, which is an important target organ for cefazolin therapy of wound infections.
MATERIALS AND METHODS

Animals and Experimental Design

Research was conducted under an approved animal use protocol in an AAALAC International-accredited facility in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 2011 edition (National Research Council (US) Committee, 2011). Forty-eight adult (12-week-old) male BALB/c mice (Charles River Laboratories, Wilmington, MA) were divided into a blast (exposed to blast overpressure of 19 pounds per square inch) and control (same procedures without blast exposure) groups. One hour after blast exposure, animals were given a bolus intravenous injection of cefazolin (328 mg/kg) (West-Ward Pharmaceuticals, Eatontown, NJ). Three mice per time-point in each group were euthanized at 3 minutes, 10 minutes, 15 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, and 10 hours post-injection. Plasma, liver, and skin were harvested for mass-spectrometry analysis.

Blast Procedure

Mice were anesthetized by inhalation of isoflurane (2.5% for 5 minutes) and subjected to a survival blast exposure of 19 pounds per square inch with an advanced blast simulator as previously described (Antonic et al., 2020). Control animals underwent the same anesthesia regimen, placement in the blast tube but without deployment of the blast overpressure.

Cefazolin Measurement

Cefazolin concentrations were determined by ultra-performance liquid chromatography-tandem mass spectrometry (Xevo TQ-s-Micro, Milford, Massachusetts). Plasma fractions were
extracted from blood samples using acetonitrile (Fisher Scientific, Pittsburgh, Pennsylvania) (2x volume) and centrifugation. Liver and skin tissue samples were homogenized in ultrapure water (5x volume) and the resulting slurry was extracted using the same method as plasma extraction.

**Pharmacokinetic Modelling**

Non-compartmental analysis (NCA) and Population PK modeling were performed in Pumas version 1.05 (Rackacukas, 2018), a Pharmacokinetic/Pharmacodynamic estimation and simulation package in the Julia programming language (Bezanson, 2017). NCA was performed by considering the data as sparse given only one data observation was available for each mouse. The first order conditional estimation method with interaction was used to estimate population parameters. Data preparation, exploratory analysis, and graphs were performed in either Pumas or R version 3.6.1 (R Core Team, 2020).

**Missing Data**

There were 6 concentration observations (all from skin) that were below the lower limit of quantification (LLOQ). Data from 17 of 48 mice (35.4%), all at collected time points prior to 1 hour, were considered missing because of dose extravasation. For NCA, concentration observations below the LLOQ were considered missing and NCA was performed using with the entire remaining data set and repeated excluding the mice with dose extravasation. Population estimates were obtained only after excluding mice with dose extravasation. Given that only one observation was available for each mouse post-dose, mean concentrations were imputed in place of the LLOQ, otherwise there would be 0 post-dose observations in those mice for the skin compartment. Primary interference was based off of the population estimates excluding mice with dose extravasation.
Plasma Model

First, the plasma data was modeled alone using a standard step-wise approach to select an appropriate base model and explore covariates. One-and two-compartment models were explored. Between-mouse variability was modeled using an exponential error model under the assumption that PK parameters are distributed log normally. Parameters generally took the form

$$\theta_i = tv\theta \cdot e^{\eta_i}$$  \( \#(1) \)

where $$\theta_i$$ is the post hoc estimated parameter value for mouse $$i$$, $$tv\theta$$ is the population mean parameter and $$\eta_i \sim (0, \omega^2)$$ is the between mouse random effects for individual $$i$$. Covariates tested in this model were blast as a categorical covariate on central volume of distribution ($$V_c$$) and terminal clearance ($$CL$$) and weight as a continuous covariate on $$V_c$$ and $$CL$$. Continuous covariates were modeled as

$$\theta_i = tv\theta \cdot \left( \frac{COV}{COV_{median}} \right)^{\theta_{COV}}$$  \( \#(2) \)

where $$\theta_i$$ is the PK parameter in mouse $$i$$, $$tv\theta$$ is the typical value of the PK parameter at the median value of the covariate ($$COV_{median}$$), $$COV$$ is the covariate observed in mouse $$i$$, and $$\theta_{COV}$$ is the power estimate for the covariate. Categorical covariates were modeled as

$$\theta_i = tv\theta \cdot (1 + \theta_{COV} \cdot COV)$$  \( \#(3) \)

where $$COV$$ is binary (coded as 0 or 1), $$tv\theta$$ represents the typical value of the PK parameter when $$COV = 0$$ and $$\theta_{COV}$$ represents the proportional change in $$tv\theta$$ when $$COV = 1$$. Selection of the base model and inclusion of covariates were based on the likelihood ratio test with $$\alpha = 0.05$$, plausibility, precision of parameter estimates, and diagnostic plots, utilizing a step-wise forward addition process.

Semi-Mechanistic Model
After establishing a base model using the plasma data alone, all the data from the plasma, liver and skin were modeled simultaneously using the plasma base model linked to liver and skin effect compartments via the central plasma compartment (Figure 1) (Gobburu et al., 2001). The amount of cefazolin in each organ compartment was modeled with the following differential equations:

\[
\frac{dA_{\text{liver}}}{dt} = K_{\text{liver}} \cdot PC_{\text{liver}} \cdot A_c - K_{\text{liver}} \cdot A_{\text{liver}} \#
\]

\[
\frac{dA_{\text{skin}}}{dt} = K_{\text{skin}} \cdot PC_{\text{skin}} \cdot A_c - K_{\text{skin}} \cdot A_{\text{skin}} \#
\]

where \( K_{\text{liver}} \) and \( K_{\text{skin}} \) are the equilibrium rate constants for the cefazolin in the liver and skin, respectively, and \( PC_{\text{liver}} \) and \( PC_{\text{skin}} \) represent the pseudo-partition coefficients between the plasma and liver and plasma and skin, respectively. The physiologic interpretation of pseudo-partition coefficients is more broadly defined than the true partition coefficient and encompass any reason (i.e., blood flow, permeability) as to why concentrations may differ from the plasma to the specified organ. Under normal physiologic conditions, the pseudo-partition coefficients approximate the true partition coefficient.

Covariates (weight and presence of blast) were tested in this model using the same definitions above. Given the sparse nature of the data (one observation in each compartment per mouse), covariates were tested primarily based on physiologic relevance and otherwise were limited to those found to be statistically significant when modeling the plasma data alone. Inclusion of covariates in this semi-mechanistic model were based on the likelihood ratio test with \( \alpha = 0.05 \) and physiologic plausibility, again utilizing a step-wise forward addition process.

**Model Qualification**

Final model qualification was based on standard goodness of fit plots, plausibility of parameter estimates and comparison of simulations to the observed data from Antonic et al. Precision of
parameter estimates was also considered, however was not a primary measure of model quality as only one observation was available from each mouse.
RESULTS

Non-Compartmental Analysis

NCA parameters are summarized in Table 1. We observed a 3.59-fold increase in blasted mice liver $AUC_{last}$ compared to controls, confirming the findings of Antonic et al. (4.3-fold increase).

Skin $AUC_{last}$ was decreased by approximately 20% in blasted mice compared to controls. Cefazolin half-life in all observed organs was similar between blasted and control mice (approximately within 20%). Maximum concentrations are consistent with Antonic et al; however, they are not comparable between blasted mice and controls in this experiment given that dose extravasation occurred at many of the early time points. Repeat NCA using all data points (including dose extravasation) resulted in similar findings (generally within 20%) with 4.1-fold increase in blasted mice liver $AUC_{last}$ compared to controls. This is within the expected variability of such traditional mouse PK studies and therefore was considered acceptable to perform nonlinear mixed-effects modeling with excluding mice with dose extravasation.

Plasma Model

Model building process and parameter estimates of the internal covariate model are summarized in Tables S1 and S2 (supplementary material). The data were best described by a two-compartment model with proportional error and blast as a statistically significant covariate on $CL$. The model was parameterized using intra-compartmental clearance ($Q$) and peripheral volume of distribution ($V_p$). The final equation for $CL$ in this model was

$$CL_i = 0.048 \cdot (1 - 0.4 \cdot isBLAST) \cdot e^{ηCL_i}$$

Semi-Mechanistic Model
Parameter estimates are summarized in Table 2 and the model building process is summarized in Table S3 (supplementary material). Variability of $V_c$, $V_p$, and $Q$ in this model was approximately 0, and were therefore no longer estimated. The typical values $PC_{liver}$ and $PC_{skin}$ were 1.14 and 0.11, respectively. These values represent the organs’ respective partition coefficients under normal physiologic conditions and are consistent with estimates in rats and rabbits (Tsuji et al., 1983; Tsuji et al., 1985). There was near instantaneous equilibration of concentrations in the liver and skin as compared to the plasma, and estimates of the corresponding parameters $K_{liver}$ and $K_{skin}$ were imprecise or caused model instability. Therefore, based on graphical interpretation and empirically testing several values, these values were fixed to $K_{liver} = 17.8 \text{ ms}^{-1}$ and $K_{skin} = 0.7 \text{ ms}^{-1}$, which correspond to near instantaneous equilibration half-lives of 1 millisecond or less. Blast was found to be a statistically significant covariate on $PC_{liver}$, with a large estimated effect of 326% increase. This can be interpreted that blasted mice had on average a 326% increase in liver cefazolin exposure as compared to control mice. We elaborate further on the physiologic mechanisms in the discussion. Of note, incorporating blast as a covariate on $PC_{liver}$ explained more of the variability on clearance (40% to 30.5% coefficient of variation) than blast as a covariate on $CL$ (40% to 34.5% coefficient of variation) (Figure S1). The final parameterization of $PC_{liver}$ using this categorical covariate model was

$$PC_{liver} = 1.14 \cdot (1 + 3.26 \cdot isBLAST)$$

In both Antonic et al. and our repeat data, the relative increase in liver concentrations in the blasted mice, when compared to the control mice, appeared to rise and fall over the 10-hour study period. We therefore explored a time-varying parameterization of $PC_{liver}$ by empirically fitting linear models to the plasma:liver concentration ratio over time to elucidate the appropriate mathematical trend and initial estimates (Figure 2). The time-varying parameterization of $PC_{liver}$ produced the best statistical model with a statistically significant improvement over the
categorical parameterization of $PC_{\text{liver}}$ ($p = 0.01$, Table S3, Model 7). The final equation for this parameterization was

$$PC_{\text{liver}}(t) = 1.14 \cdot (1 + isBLAST \ast (2 \cdot t - 0.18 \cdot t^2))$$

where $PC_{\text{liver}}(t)$ is the pseudo-partition coefficient at time $t$. Although this model better described the data, it is more difficult to interpret and the ability to extrapolate is unclear; hence, we present both models.

Before accounting for blast effect on $PC_{\text{liver}}$, there was an apparent statistically significant 66% decrease in $PC_{\text{skin}}$ in blasted mice compared to controls. However, after accounting for blast effect on $PC_{\text{liver}}$, the effect of blast on $PC_{\text{skin}}$ was no longer statistically significant and the effect size was far less (29.8% decrease). This was more consistent with NCA based estimates (approximately 20% decrease of skin AUC in blasted mice compared to controls). Given this blast effect on $PC_{\text{skin}}$ was not statistically significant, it was not included in the final model.

**Model Qualification**

Plasma and liver diagnostic plots (Figures 3 and S2, respectively) appear without major trends suggesting no model misspecification of these compartments. The skin diagnostic plots (Figure S2) demonstrated minor trends, suggesting possible skin model misspecification; however, given the sparse nature of the data and desire to utilize the simplest model (rather than a full physiologic based pharmacokinetic model), these trends were considered acceptable.

Histograms and quantile-quantile plots of random effects of $CL$ (figures S4 A and B) and conditional weighted residuals of the plasma, liver and skin compartments (figures S5 A and B) generally follow a normal distribution centered around 0. Of note, there was some skew off the line of unity in the liver conditional weighted residuals quantile-quantile plot, and suggestion of bimodality. However, again this was considered acceptable given limitations of the dataset.
Simulated typical time concentration profiles of both plasma and liver data in blast and control mice are highly consistent with the observed data from Antonic et al. (Figures 4 A and B).
DISCUSSION

We confirmed the findings of Antonic et al. by demonstrating that blast is associated with a 3- to 4-fold increase in liver cefazolin exposure and extended the previous research by including skin cefazolin concentrations and developing a semi-mechanistic population model. Blast exposure was a statistically significant covariate on $PC_{\text{liver}}$, with an average 326% increase in liver cefazolin concentrations over the 10-hour study period. This covariate also explained 8.5% variability of the systemic cefazolin clearance predicting slightly higher concentrations of plasma cefazolin in blasted mice compared to controls. Finally, we observed a non-statistically significant decrease (20 – 30%) in cefazolin skin exposure as measured by AUC or covariate effect on $PC_{\text{skin}}$.

Regional changes in blood flow following blast exposure most likely explain these findings, though altered permeability and protein binding may also contribute. The pseudo-partition coefficient encompasses any physiologic mechanism beyond permeability that may explain differences in plasma and end-organ concentrations. Under normal physiologic conditions, the pseudo-coefficient approximates the true partition coefficient, as seen with our parameter estimates for $PC_{\text{liver}} = 1.14$ and $PC_{\text{skin}} = 0.11$ that are consistent with estimates in both rat and rabbit PK studies (Tsuji et al., 1983; Tsuji et al., 1985).

Blast overpressure leads to activation of the cardiac Bezold-Jarisch reflex followed by a global sympathetic response (Wolf et al., 2009; Committee on Gulf et al., 2014). Sympathetic activation leads to splanchnic vasoconstriction and arterial constriction shunting blood preferentially to the liver and central circulation while decreasing peripheral perfusion (Rutlen et al., 1979). Antonic et al. demonstrated that there was no liver or kidney damage in blasted mice via lab and histological investigation, making alteration of cefazolin terminal clearance unlikely. Further evidence supporting this hypothesis is derived from a mouse model of paracetamol-induced hepatotoxicity. Dosing mice with $\alpha_1$-adrenoceptor antagonists prevented liver injury by
countering catecholamine-induced vasoconstriction of the hepatic vasculature with subsequent decrease in liver paracetamol concentrations (Randle et al., 2008). Therefore, the observed 3.26-fold increase in $P_{C_{\text{Liver}}}$ and 45% increase in blasted mouse plasma concentrations are well explained by altered perfusion.

Altered perfusion does not exclude the possibility of increased liver permeability as a result of blast injury. A rabbit blast model demonstrated microvessel injury in the lungs, liver, and kidneys causing time-dependent hemoconcentration and increased vascular permeability as measured by leakage of radiolabeled albumin (Zhang et al., 2011). Similarly, Antonic et al. reported lung hemorrhage among the mice exposed to blast. Cefazolin is 80 – 90% protein bound, but this percentage may decrease at high cefazolin concentrations where protein binding may become saturated (Vella-Brincat et al., 2007). Hypoalbuminemia or alterations in protein binding may result in a greater proportion of free cefazolin in the plasma, driving tissue penetration in liver and skin. However, several findings suggest that altered perfusion in the blasted mice is the primary driver of the increased liver concentrations, rather than increased permeability or altered protein binding. Antonic et al. demonstrated that there was no decrease in serum albumin resulting from the blast exposure, so free cefazolin concentrations were not likely to be significantly affected. Secondly, skin cefazolin exposure decreased by 20 – 30%. However, decreased protein binding should have led to increased skin cefazolin exposure. Therefore, mechanistically, the differential changes in liver and skin cefazolin concentrations are better explained by splanchnic blood mobilization and shunting of blood to the liver accompanied with capillary vasoconstriction decreasing skin perfusion. Furthermore, the ratio of liver to plasma concentrations changed rapidly over time in blasted mice, peaking around 6 hours and returning to baseline by the end of the experiment. This is better explained by transient alterations in organ perfusion, as capillary damage would likely take longer than 10 hours to normalize. While our results did not reach the level of statistical significance in skin,
likely due to small number of samples per time point, our results suggest a potential contributory role of blast on cefazolin exposure in target tissues.

A final mechanistic consideration is inflammatory changes. Blast exposure may injure tissue and increase inflammation (Committee on Gulf et al., 2014). Inflammation downregulates and inhibits drug metabolizing enzymes and transporters, such as CYP3A4 and p-glycoprotein, and may alter plasma protein binding (Seifert et al., 2017). However, cefazolin is almost exclusively cleared by the kidney and is not a known p-glycoprotein substrate, making inflammation unlikely to contribute to changes in cefazolin PK.

This study brings up important questions regarding the treatment of wounded Service Members. Among patients with combat-related extremity wound infection, epidemiological data shows that blast exposure is significantly correlated to infections (Stewart et al., 2019). Still, Service members may be exposed to significant blast overpressure when conducting breaching operations or using heavy weapons (Carr et al., 2016; Skotak et al., 2019). Tourniquets reduced tissue concentrations of antibiotics to affected limbs, even days after release in a rat model (Mangum et al., 2019). Thus, the use of tourniquets in in blast-injured Service Members may result in failure of systemically delivered antibiotics to injured tissues. A combined injury model is currently in development. However, blast effect on cefazolin PK appears transient and it is unclear how this effect would scale to humans. At this time, we do not recommend any dosing changes for a similar injury in humans in the absence of measurable kidney damage. Another clinical consideration is the possibility of drug-induced liver injury (DILI). While there was no evidence of liver injury in these mice at the time points of investigation, there is growing evidence of cefazolin associated with DILI (Alqahtani et al., 2015). Alqahtani et al. reported 19 patients who developed transient, mostly moderate-severe DILI after a single dose of cefazolin. It is conceivable that increased cefazolin liver concentrations associated with blast injury may make patients more susceptible to cefazolin DILI.
Limitations of the study include missing data due to dose extravasation (35.4%) as well as the inability to collect repeated plasma measurements for each mouse. Despite the significant proportion of missing data, all occurred before 1 hour, allowing adequate terminal half-life data for PK estimation. Further, we were able to accurately predict plasma cefazolin concentration data from Antonic et al. at time points before 1 hour, suggesting that our model is still externally valid despite the missing data. Additionally, when including all data, NCA demonstrated a stronger trend of increased liver AUC (310% increase vs 258% increase) and decreased skin AUC (29% decrease vs 3% decrease), consistent with the known physiologic changes to perfusion following blast exposure.

Having only one set of organ concentration observations per mouse contributed to the low precision of some estimates. Inference-based confidence intervals were not obtainable so we relied on bootstrap estimation for parameter precision. When a mouse provides only one concentration point, that mouse may be resampled multiple times effectively altering the PK curve for that bootstrap dataset. However, all parameter estimates were plausible; diagnostic plots suggested that the model adequately describes the data internal to this experiment and that a 2-compartment model was the best statistical model consistent with literature based on rich data collection (Cagnardi et al., 2018).

Cefazolin liver concentrations in our study were on average a log$_{10}$-fold higher than in Antonic et al., despite having highly consistent plasma cefazolin concentrations in both the current and prior studies. We strongly suspect an accidental dilution of liver concentrations in Antonic et al., as nearly the exact same proportional increase in liver concentrations over the same time-course were observed in our study. Further, the estimated liver partition coefficient from control mice in Antonic et al. was extremely low at 0.13, which is lower than the 95% confidence interval observed in prior literature and our repeat study.
Conclusions

We have established a semi-mechanistic model of cefazolin PK based on the two-compartment model with liver and skin effects compartments with validation using data from the previous experiment. Blast was a significant covariate on the liver pseudo-partition coefficient, increasing liver concentrations relative to plasma by 326%. The likely physiologic explanation is altered global perfusion starting with an initial vasovagal reaction followed by compensatory vasoconstriction of the splanchnic and peripheral circulations. Plasma concentrations were not affected in a clinically meaningful way, and the effect of blast on liver concentrations is large but transient. Although it is unclear how blast will affect human physiology over time, it is unlikely that any dosing changes of cefazolin would be required specific to blast overpressure exposure. However, clinicians should have a higher suspicion for cefazolin DILI in patients suffering from blast overpressure injury.
ACKNOWLEDGEMENTS

We would like to thank Ms. Zanete Wright for her devoted work to the USU/WRAIR Clinical Pharmacology Fellowship. This research would not have been possible without her help.
AUTHORSHIP CONTRIBUTIONS

Participated in Research Design: Antonic, Chin, DeLuca, Getnet, Livezey

Conducted Experiments: Bobrov, Sajja

Contributed New Reagents or Analytic Tools: Long, Swierczewski, Tyner

Performed Data Analysis: Chin, Selig

Wrote or contributed to the writing of the manuscript: Antonic, Bobrov, Chin, DeLuca, Getnet, Livezey, Long, Sajja, Selig, Swierczewski, Tyner
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FOOTNOTES

No author has an actual or perceived conflict of interest with the contents of this article. This work was supported by the Military Infectious Disease Research Program (grant numbers W306_19_WR and W0338_20_WR). Material has been reviewed by the Walter Reed Army Institute of Research and the Uniformed Services University of the Health Sciences. There is no objection to its presentation and/or publication. The views expressed in this article are those of the authors and do not reflect the official policy or position of the U.S. Army Medical Department, Department of the Army, DoD, or the U.S. Government.
FIGURE LEGENDS

Figure 1. Model Structure. Two-compartment pharmacokinetic model with liver and skin effect compartments. CL is the terminal clearance, Q is the intra-compartmental clearance, $V_c$ is the volume of the central compartment, $V_p$ is the volume of the peripheral compartment, $PC_L$ is the pseudo-partition coefficient of the liver, $PC_S$ is the pseudo-partition coefficient of the skin, $K_L$ is the equilibrium constant of the liver, and $K_S$ is the equilibrium constant of the skin.

Figure 2. Liver Plasma Ratio Trends over Time. Liver:Plasma concentration ratio (LPR) over time in the blasted (black) and control (green) mice. Black and green dashed trend lines represent linear models fitting the blasted and control mice, respectively.

Figure 3. Plasma Goodness of Fit Plots.

Figure 4. External Validation of Model. External Validation of model using data from Antonic et al. as a validation dataset. Left (A) represents plasma concentration data and right (B) represents liver concentration data. Solid lines represent mean simulations and closed circles represent data from Antonic et al. The solid black line in figure B represents simulations of blasted mice utilizing the categorical parameterization of $PC_L$, where the dashed black line in figure B represents simulations of blasted mice utilizing the time-varying parameterization of $PC_L$. We strongly suspect there was an accidental dilution in determining liver concentrations in Antonic et al. and therefore these liver concentrations are assumed to be 10-times higher than reported. See discussion for further details.
### Table 1: Non-Compartmental Analysis Parameter Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma</th>
<th>Liver</th>
<th>Skin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Blast</td>
<td>Control</td>
</tr>
<tr>
<td>$AUC_{last}$ (mg*hr/L)</td>
<td>197.97</td>
<td>287.21</td>
<td>233.07</td>
</tr>
<tr>
<td>$C_{max}$ (mg/L)</td>
<td>355.03</td>
<td>185.12</td>
<td>574.07</td>
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<tr>
<td>$T_{1/2}$ (h)</td>
<td>0.85</td>
<td>0.93</td>
<td>0.97</td>
</tr>
</tbody>
</table>

$AUC_{last}$ is the Area Under the Curve using the last sampled time point, $C_{max}$ is the maximal concentration, and $T_{1/2}$ is the terminal elimination half-life.
Table 2: Pharmacokinetic Parameters for Final Model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (Bootstrap %RSE)</th>
<th>Bootstrap 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CL$ (L/h)</td>
<td>0.048 (27.8)</td>
<td>0.01 – 0.062</td>
</tr>
<tr>
<td>$V_c$ (L)</td>
<td>0.039 (25)</td>
<td>0.012 – 0.055</td>
</tr>
<tr>
<td>$Q$ (L/h)</td>
<td>0.0022 (&gt; 672.8)</td>
<td>0.00093 – 0.062</td>
</tr>
<tr>
<td>$V_p$ (L)</td>
<td>0.0066 (&gt; 1000)</td>
<td>0.0026 – 43.33</td>
</tr>
<tr>
<td>$PC_{liver}$</td>
<td>1.14 (14.8)</td>
<td>0.84 – 1.34</td>
</tr>
<tr>
<td>$PC_{skin}$</td>
<td>0.11 (23.4)</td>
<td>0.067 – 0.17</td>
</tr>
<tr>
<td>$K_{liver}$ ($ms^{-1}$)</td>
<td>17.8 fixed</td>
<td>-</td>
</tr>
<tr>
<td>$K_{skin}$ ($ms^{-1}$)</td>
<td>0.7 fixed</td>
<td>-</td>
</tr>
</tbody>
</table>

**Covariates**

- Blast on $PC_{liver}$ (categorical 1+)
  - $B_1$ = 2
  - $B_2$ = 0.18

**Random Effects**

- $\omega^2 CL$ = 0.093 (> 1000)  
  - 0.015 – 0.69

- $\eta$-shrinkage $CL$: 13.7%

**Residual Unexplained Variability**

- Proportional Error Plasma = 0.5 (39.1)  
  - 0.29 – 0.67

- Proportional Error Liver = 0.6 (14.6)  
  - 0.42 – 0.75

- Proportional Error Skin = 0.61 (14.3)  
  - 0.41 – 0.76

$\epsilon$-shrinkage plasma: 8.9%, $\epsilon$-shrinkage liver: 6%, $\epsilon$-shrinkage skin: 7.5%

93 total observations, 31 in each plasma, liver, and skin. Each mouse provided a single observation for each of the plasma, liver, and skin compartments. Objective Function Value = 889.12.
Figure 1. Model Structure
Figure 2. Liver Plasma Ratio Trends over Time
Figure 3. Plasma Goodness of Fit Plots
Figure 4. External Validation of Model