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Title: Accelerated repurposing and drug development of pulmonary hypertension therapies for COVID-19 treatment using an AI-integrated biosimulation platform

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Abstract

A World Health Organization-declared pandemic, COVID-19, has affected more than 4 million people worldwide with over 100,000 deaths and growing in the United States. Due to the fast-spreading and multi-targeted nature of the virus, it is clear that drugs and/or vaccines need to be developed at an accelerated rate, and a combinatorial approach may stand to be more successful than a single drug therapy. Among several targets and pathways that are under investigation, the renin-angiotensin system (RAS) and specifically Angiotensin converting enzyme (ACE), and Ca²⁺-mediated SARS-CoV-2 cellular entry and replication are noteworthy. A combination of ACE inhibitors (e.g. benazepril) and calcium channel blockers (CCB, e.g. amlodipine), a critical line of therapy for pulmonary hypertension, has shown therapeutic relevance in COVID-19 when investigated independently. To that end, we conducted *in silico* modeling using BIOiSIM, an AI-integrated mechanistic modeling platform by utilizing known preclinical *in vitro* and *in vivo* datasets to accurately simulate systemic therapy disposition and site-of-action penetration of the CCB and ACEI compounds to tissues implicated in COVID-19 pathogenesis.

Keywords: COVID 19; ACE inhibitors; Calcium Channel Blockers; Artificial Intelligence; Drug Development; Drug Repurposing

INTRODUCTION

COVID-19 Epidemiology

The emerging pandemic of COVID-19, affecting millions worldwide, presents an extraordinary challenge to identifying effective drugs for prevention and cure ^{6,7}. Analysis of the cumulative surveillance data in the last few months, showed 5,817,385 reported cases and 362,705 deaths worldwide and including 1,761,503 aggregated reported cases and 103,700 deaths in the United States ⁸. Although average daily mortality rates are declining, 7-day moving averages of daily incidence of COVID-19 cases indicate ongoing community transmission ⁸. Prevention and containment of the outbreak is paramount, contingency measures with experimental therapeutics are being urgently investigated for the immediate unmet medical need.

COVID-19 Therapies; Key Challenges and Current Therapies

There are several monotherapies (i.e. remdesivir, camostat) being tested for COVID-19, but unfortunately, they have yet to demonstrate significantly high efficacy rates over placebo as well as lower mortality (e.g. hydroxychloroquine) [4]. The current pipeline for therapeutic interventions for COVID-19 consists largely of older antivirals, immunomodulatory agents, and traditional Chinese medicines (Table 1); currently, no COVID-19 therapeutic options are available or approved other than supportive care measures. Hence, a multitargeted approach may have a greater potential to be a more successful therapy than a single drug target due to the multifactorial-polygenic infectious nature of the virus. In this critical scenario, development of a vaccine or an antiviral medication is a promising approach but is unlikely to be completed for several years. Currently, the most advanced therapy in development is remdesivir ⁹. This antiviral drug was seen to be more efficacious to placebo in shortening the time to recovery in adults hospitalized with Covid-19 ¹⁰, but it is not US FDA approved and is currently being tested in randomized trials.

Compound Name	Development	Overview	Source
Remdesivir	Gilead, Phase III in China/Asia & US	Antiviral, host factor–targeted. RNA-dependent/RNA polymerase–targeted	¹¹
APN01	APEIRON Biologics, Phase I	Pilot trial ongoing in China	⁹
Brilacidin	Innovation Pharmaceuticals, Phase II	Defensin mimetic drug candidate. Has shown antibacterial, anti-inflammatory, and immunomodulatory properties in several clinical trials	¹¹
Hydroxychloroquine	Repurposed	Host factor–targeted. Antimalarial drug that affects endosomal function and blocks autophagosome-lysosome fusion	¹²
Azithromycin	Repurposed	Host factor–targeted. Broad-spectrum antibiotic, blocks autophagosome clearance in human cells	¹³

Camostat	Repurposed	Host factor–targeted. TMPRSS2 inhibitor	14
Nafamostat	Repurposed	Host factor–targeted. TMPRSS2 inhibitor	15
Favipiravir	Repurposed	Host factor–targeted. RNA-dependent/RNA polymerase–targeted	15

Table 1: Select compounds currently in clinical trials for treating COVID-19

There is a race to develop effective interventions since COVID-19 cases are increasing daily. While intensive research and clinical trials to address this critical unmet need, are being developed to determine the efficacy of known drugs and identify potential therapeutic targets to develop new drugs for treating COVID-19, results to date on the efficacy of the drug are inconclusive and inconsistent and safety profiles are unknown in the context of the disease ¹⁶. Recently, a number of other drugs are also being considered such as combinations of ACE inhibitors and CCBs for development ⁹. Their anti-inflammatory, anti-fibrotic and vasodilatory roles have been well established in the pathophysiology of pulmonary hypertension (PH), a chronic health condition recognized as a high risk factor in severe COVID-19 disease ¹⁷. ACE inhibitors and CCB, used in the treatment for PH, have shown therapeutic efficacy in COVID-19 ^{18,19}, when investigated separately. Hence, it can be postulated that key proteins pertaining to the renin–angiotensin signaling system (RAS) and indirectly acting via ACE2, such as ACE, can be potential targets to combat viral replication and the post-infection pro-inflammatory responses known as the "cytokine storm".

SARS-CoV-2 virus & infection

The growing knowledge regarding SARS-CoV-2 pathophysiology provides a significant number of potential drug targets, specifically focused on the virion structure and target tissues. SARS-COV-2 is a large (27–32 kb), enveloped, positive-stranded RNA virus. It consists of four proteins: membrane protein (M), envelope protein (E), spike protein (S), and nucleocapsid protein (N) ²⁰. The spike protein facilitates the entry of the virus into host cells ²⁰ and it is a critical factor of viral host range and tissue tropism and is a major inducer of host immune response and disease severity ^{19,21,22}. Viral spike protein is involved in receptor binding and subsequent viral entry into the host cells ²¹. At least initially, it binds to the cellular receptor angiotensin converting enzyme 2 (ACE2), cellular receptor TMPRSS2, and the calcium channel post entry in nasal secretory goblet cells, lung type II pneumocytes and gut absorptive enterocytes ²³. Subsequently, these proteins pertaining to the renin-angiotensin signaling system (RAS) can be considered as potential therapeutic targets.

Targeting of ACE in RAS for the Attenuation of the Pro-Inflammatory response Post Infection: ACE Inhibitors

ACE, a zinc-metalloproteinase, converts Angiotensin (Ang) I to Ang II, Ang II mostly exerts its activity via a type 1 and type 2 (AT1 and AT2) receptor maintaining blood pressure homeostasis, and anti-inflammatory response in addition to salt and fluid balance ²⁴. ACE2 generates Ang (1-7) from Ang II, Ang (1-7) after binding and activating the mitochondrial assembly 1 (MAS) receptor broadly, shifts the balance from vasoconstriction with Ang II to vasodilation ²⁵. The role of this vasodilatory effect in the pathogenesis of COVID-19 remains unclear, but some animal data suggest a beneficial effect in PH ²⁵. Additionally, ACE2 and Ang (1-7) have been found to be protective in several different lung injury models ^{25,26}. ACE inhibitors may potentially attenuate the COVID-19 associated "cytokine storm" by upregulating ACE2, which converts Ang II to Ang (1–

7), activation of ACE2, and the downstream MAS receptor has beneficial vasodilatory and anti-inflammatory properties that could play a potential role post-infection of COVID-19 ²⁷.

Targeting Ca²⁺ Channel for The Reduction of Viral Entry and Replication: CCBs

The underlying mechanism of action of CCBs on SARS-CoV-2 needs elucidation and remains unclear to date. Several pathogenic viruses induce intracellular calcium influx by hijacking predominantly the voltage-gated Ca²⁺ channels (VGCC), to facilitate viral entry, replication, and prolonged infection ^{28,29}. In previous studies with Ebola and similar classes of virus ^{30,31}, CCBs displayed inhibition of the replication of viruses after entry. Case Fatality rates were markedly reduced with CCB treatment among patients that were infected with Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV) ³¹. Similarly, to SFTSV patients, CCBs are postulated to interfere with SARS-CoV-2 replication after cellular entry. The putative mechanism of action of CCBs entails the interference with the intracellular calcium influx instigated by the virus and blocking of calcium-dependent signaling pathways pivotal to viral replication. Subsequently, CCBs may act as host-signaling targeted compounds that reduce the rate of viral mutations and interferes with the replication process via modulation of virus-hijacked host cellular machinery, compared to antivirals which target many viral proteins ³². As a result, this could be an important factor in the development of compounds against SARS-CoV-2, an RNA virus with a potentially high mutation rate ³². Additionally, a key consequence of viral pathogenesis is marked by a strong inflammatory response preceded by a set of sequentially activated signaling pathways such as increased intracellular calcium levels leading to mitochondrial dysfunction and eventually cellular apoptosis ³³⁻³⁵. In prior clinical outcomes, CCBs attenuated markedly the proinflammatory response by modulating the intracellular calcium levels to homeostasis in patients and reduced death rates in septic animal models with the high systemic proinflammatory state ^{36,37}. Additionally, the global attenuation of proinflammatory cytokines and oxidative stress by CCBs was observed in hypertensive patients compared to baseline ³⁸. It can be hypothesized that CCBs, besides interfering with viral replication, may attenuate systemic inflammatory responses in patients to impart the clinical benefits synergistically with their antiviral efficacy. These therapies may be particularly relevant to COVID-19 given it is associated with an extended proinflammatory state in patients ³⁹.

Combinatorial Effect of ACE inhibitors and CCBs as a potential treatment for COVID 19

Combinatorial approaches using ACE inhibitors, acting in an anti-inflammatory fashion, and CCBs countering the virus post-entry-stage and re-establishing Ca²⁺ homeostasis and consequently down-regulating the pro-inflammatory signals, may impart synergistic outcomes in increasing the clinical efficacy of reducing viral load in the patient, compared to individual drug treatments (Figure 1). ACE inhibitors may potentially attenuate the COVID-19 associated "cytokine storm" by upregulating ACE2, which in turn imparts beneficial vasodilatory and anti-inflammatory properties ^{27,40} (Figure 1). Several pathogenic viruses induce intracellular calcium influx to facilitate viral entry and infection ^{28,29}. In previous studies with Ebola and Severe fever with thrombocytopenia syndrome (SFTS) virus ³¹, CCBs inhibited the replication of viruses, post-entry ³⁰. Similarly, CCBs are postulated to inhibit the post-entry events of COVID-19 replication ³¹. It is hypothesized that CCBs block the virus-induced intracellular calcium influx and impair calcium-dependent cellular pathways that are critical for virus replication ³², and subsequently, strong inflammatory responses ³³⁻³⁵. Combinations of ACE inhibitors and CCBs have been used in the past for the treatment of PH ⁴¹ and may impart synergistic efficacy in reducing viral load and associated inflammation in COVID-19 patients. However, there is insufficient data on ACE/CCB therapies available to verify whether these observations are translatable to humans, and no studies have evaluated the effects of combinations of RAS inhibitors and CCBs in COVID-19 trials.

Figure 1: Potential model for the combinatorial actions of ACEi and CCBs on SARS-CoV-2 infection, replication and pro-inflammatory response. ACEi acting indirectly through ACE2 and the MAS/ATR2 axis imparts an anti-inflammatory and anti-fibrotic response, while CCBs acts at various steps, restores intracellular calcium flux, consequently, inhibiting post-infection virus internalization and genome replication. Both ACEi and CCBs potentially can induce a synergistic anti-inflammatory and anti-fibrotic response in the attenuation of the "Cytokine storm".

BIOiSIM Platform

Here, we apply BIOiSIM, an AI/ML-based PK-PD modeling platform in the repurposing of ACE inhibitors and CCBs for generating predictions of compound disposition in the context of COVID-19. It is a dynamic, biology-driven platform that provides a scalable computational prediction of *in vivo* pharmacokinetic-pharmacodynamic (PK-PD) phenomena. A potential treatment strategy for COVID-19 will consist of a two-pronged approach; initially, minimizing the rate and extent of *in vivo* SARS-CoV-2 infection/replication and second, reducing the systemic inflammatory effect implicated in the "cytokine storm" post-infection. We aim to use the BIOiSIM platform to conduct *in silico* modeling of CCB/ACE inhibitor combinations integrate known preclinical *in vitro* dataset and simulation of *in vivo* drug therapy disposition and simulate disposition to relevant tissues to accelerate targeted therapy development for COVID-19.

RESULTS

Sensitivity and Convergence Testing

For cases where parameter values were not available experimentally, BIOiSIM's optimization algorithms were utilized to obtain parameter values enabling simulation of drug disposition in both the plasma venous compartment as well as the COVID-19-associated sites-of-interest. Figure 2 highlights these outcomes by showing how the objective function (cost) varies over the trials that were tested during the coarse grid search phase of optimization. There is confidence in the optimized parameter values because of the clear absolute minima that can be visualized looking at the convergence plots for Verapamil, Spirapril, Lisinopril, Lacidipine, and Captopril (highlighted with an arrow). The final optimized values are summarized in Table 3. Table 4 gives an overview of the datasets used for the study.

Simulation Accuracy

BIOiSIM simulations of plasma concentration over time relative to experimental data are captured in Figure 3¹⁻⁵. The performance of the core model across the different compounds was assessed using a combination of AFE and AAFE values across the metrics generated from non-compartmental analysis and chi-squared hypothesis testing. Additionally, visual analysis of the simulation fits against the validation data was conducted to assess goodness-of-fit, and served as a key metric in assessing the platform performance in cases where error/variability data was not available from the original source. As highlighted in Table 2, the AAFE for $t_{\max}$, $C_{\max}$, and AUC_{0-t} for all of the compounds – Lisinopril, Captopril, Spirapril, Lacidipine, and Verapamil - was less than 2, indicating that the simulation results were close to recapitulating the actual *in vivo* behavior. The average AFE values for the compound metrics (1.03, 0.92, and 1.08 for AUC_{0-t} , $C_{\max}$, and $t_{\max}$, respectively) are within fold-error of ± 0.1 and indicate minimal bias toward systematic over- or under-prediction of output values. Both IV and orally administered compounds were simulated with similar accuracy.

Visual analysis of the plots is also indicative of overall high accuracy, with the simulation outputs accurately captured the ascending and terminal phase that aligns with the expectation for IV and Oral routes-of-administration. For Lacidipine and Captopril, the two compounds where data was available for multiple doses, the maintained accuracy of simulation outputs (AAFE < 1.3 across all metrics) supports the model's ability to accurately simulate clearance and absorption after oral administration of the compounds. The chi-squared metrics and associated p-values did not show statistically significant fits; as referenced in Table 2, the majority of publications reviewed for *in vivo* data did not contain timepoint-specific variability, and assumptions made regarding variability are likely too conservative and result in a failure to reject the null hypothesis of difference in the plots.

Simulating Distribution to Gut, Lung, and Nasal Epithelium

After successfully validating the systemic plasma PK across compounds, predictive models generated outputs of expected tissue concentration profiles in the three COVID-implicated tissues – gut, lung, and nasal epithelium (Figure 4). One interesting observation from these outcomes is that the effective lag time in $t_{\max}$ between the nasal epithelium and the other tissues is minimal, which may indicate that permeability to the site-of-action is an insignificant rate-limiting factor. Additionally, the relative $C_{\max}$ is <2-fold different at the nasal epithelium relative to other tissues, and indeed, the other tissues are also similar to plasma levels specifically for Spirapril, Lisinopril, and Captopril. These compounds may therefore be better candidates for rapid repurposing, given that their systemic plasma profiles are likely to have data around interaction with Ca^{2+} and ACE in PH patients, and those levels are directly correlated to those in tissue.

Table 2: Comparison of Model Predicted Outputs to Experimental Outputs.

	Compounds	Lisinopril	Captopril			Spirapril	Lacidipine		Verapamil
Output Metric	ROA	Oral	IV	IV	IV	Oral	Oral	Oral	IV
	Dose (mg)	20	2.78	5.67	11.40	25	2	4	5
AUC (0-t) ($\mu\text{g}\cdot\text{h/L}$)	Observed	N/A	N/A	N/A	N/A	N/A	3.66	7.66	N/A
	Calculated	752.00	42.97	93.93	215.69	991.83	3.12	6.80	703.03
	Predicted	823.50	49.16	92.55	212.12	977.60	3.27	8.80	494.26
	AAFE	1.10	1.14	1.01	1.02	1.01	1.05	1.29	1.42
	AFE	1.10	1.14	0.99	0.98	0.99	1.05	1.29	0.70
Cmax ($\mu\text{g/L}$)	Observed	N/A	N/A	N/A	N/A	430.00	1.24	3.09	N/A
	Calculated	57.40	104.64	234.55	454.71	378.00	1.17	2.87	1176.82
	Predicted	53.93	105.81	183.46	497.25	196.17	1.00	2.01	1696.96
	AAFE	1.06	1.01	1.28	1.09	1.93	1.16	1.43	1.44
	AFE	0.94	1.01	0.78	1.09	0.52	0.86	0.70	1.44
Tmax (h)	Observed	N/A	N/A	N/A	N/A	0.90	1.13	1.25	N/A
	Calculated	6.04	0.15	0.19	0.13	1.00	1.23	1.05	0.09
	Predicted	6.03	0.15	0.19	0.13	1.75	1.05	1.05	0.09
	AAFE	1.00	1.00	1.00	1.00	1.75	1.17	1.00	1.00
	AFE	1.00	1.00	1.00	1.00	1.75	0.86	1.00	1.00
	Chi-squared (χ^2)	2803.13*	22.56	23.01	26.85	2.70	107.9*	182.15*	11.57*
	p-value	>0.50	>0.50	>0.50	>0.50	<0.001	>0.50	>0.50	0.36

Note: outputs marked as “observed” are those that were pulled directly from the source validation data manuscripts. “Calculated” corresponds to recalculation of output values using internal non-compartmental methods. Predicted C_{max} values correspond to the maximum sampling concentration within the time range of observed timepoints. For observed datapoints with poorly visible error bars, an effective error was calculated using the variability from other timepoints.

*Chi-squared values and associated P-values were calculated assuming a standard deviation of 0.1x in observed values for manuscripts without reported measurement error.

Sensitivity and Convergence Testing

Figure 2: Convergence onto discrete parameter combinations during coarse optimization for Verapamil, Spirapril, Lisinopril, Lacidipine, and Captopril. Relative cost on the y-axis is calculated as: $Cost_relative = AAFE_{C_{max}} * AAFE_{t_{max}} * AAFE_{AUC}$ where $AAFE_{C_{max}}$, $AAFE_{t_{max}}$, $AAFE_{AUC}$ – absolute average fold error for C_{max} (maximum plasma concentration), T_{max} (time corresponding to C_{max}) and AUC (area under the curve) predictions

Figure 3: Dose and route-of-administration dependent prediction of compound plasma concentration for Verapamil, Spirapril, Lisinopril, Lacidipine, and Captopril. Dashed lines correspond to BIOiSIM simulation outputs. Error bars and individual data points were digitized from the original publications ¹⁻⁵ (citations in output table 4) where available, and correspond to standard error/standard deviation, as presented in the original work.

Figure 4: Prediction of the site-specific distribution of Verapamil, Spirapril, Lisinopril, Lacidipine, and Captopril to tissues implicated in COVID-19 replication and pathogenesis.

DISCUSSION

The disposition and dosing regimen simulation of two classes of physicochemically distinct small molecules, ACEI and CCBs, were successfully simulated using BIOiSIM. The approach taken serves as initial validation of the platform for rapid repurposing of therapeutics. Specifically, this lays groundwork for a potential repurposing pipeline outside of COVID-19 and inclusive of any unmet medical need where existing drugs may target a pathway relevant to the condition. The general workflow of scanning a database of compounds, identifying accurately simulated datasets, and using proprietary optimization algorithms filled in knowledge gaps by optimizing missing experimental information within the bounds of physiological expectations enables the extension of simulation results beyond experimentally measured data and into case-specific prediction.

Figure 5: Diagram of modeling approach taken that extends beyond pharmacokinetic modeling of compound disposition and can explore more complex viral & combination effects.

Further extension and application of these results will enable greater insight into the potential therapeutic effects of ACEI and CCB combinations, as highlighted in Figure 5. One example includes pharmacodynamic models relating the extent of ACE inhibition to Ang II levels (postulated to play a role in the "cytokine storm" secondary to COVID-19) would drive hypothesis-driven modeling and simulation of patient outcomes. Additionally, optimal dosing regimens of the compounds can be derived by exploring the expression levels of Ca²⁺ channels and ACE across the relevant tissues and extrapolating from *in vitro* studies of channel blocking and inhibition to assess the likelihood that a sufficient concentration of drug can reach a site-of-action to either minimize viral replication or reduce inflammatory effects. The levels of such endogenous mediators can be linked to the expected drug concentrations in plasma or active site to gain a deeper understanding of the interaction of drug with the active site components and their downstream pharmacological effects – integration of these PK-PD parameters with VSL's BIOiSIM platform can extend the prediction of drug disposition and safety and efficacy applicable to other drug therapies in healthy and diseased populations.

As more data is collected, and the pathophysiology of COVID-19 in healthy and diseased populations is understood, more customized prescriptions of specific CCB/ACEI combinations can be derived by looking at susceptibility to drug-drug interaction and variability in relevant gene

expression levels between subjects. Overall, these results give additional confidence in the BIOiSIM platform's ability to rapidly identify and simulate drug disposition for compounds that may be efficacious therapies for rapidly emerging, deadly conditions.

MATERIALS & METHODS

Overview of BIOiSIM platform

The core functionality of the *in silico* simulation platform described here was outlined in a recent work around modeling penetration of transdermal formulations to systemic circulation [40]. Briefly, the platform is comprised of a 16-organ model of compound PK, validated across an internal database of small molecule compounds. Auxiliary models (specific PK or PD) can be integrated into the centralized framework to expand the ability to make specific predictions of compound disposition. AI-ML algorithms are utilized to either optimize missing parameters in the case of insufficient experimental data, or as predictive solutions to train on existing *in vivo/in vitro* datasets. The software systems are hosted on Amazon Web Services (AWS) cloud and enable high throughput of parallelized PK simulations (PK simulator performance is ~0.08s to simulate 1hr of drug exposure at 0.36s resolution).

The drug-dependent parameters used in the model are either experimentally determined or predicted/optimized using a combination of ML cost minimization algorithms; these include integrating iterative optimization algorithms with random walk methods (similar to Markov Chain/Monte Carlo) to converge on a global minimum. Details of the approach are discussed in our recent publication ⁴².

Modeling compound disposition

Computing the mass balance between the compartments follows the general form:

$$V_{organ} \frac{dC_{organ}}{dt} = Q_{organ}(C_{blood,in} - \frac{C_{organ}}{K_{T:P,unbound} * f_{unbound,plasma}} * B:P) \quad (1)$$

For metabolizing and eliminating organs (e.g. liver, kidney) the general relationship in the model is defined as:

$$V_{organ} \frac{dC_{organ}}{dt} = Q_{organ}(C_{blood,in} - \frac{C_{organ}}{K_{T:P,unbound} * f_{unbound,plasma}} * B:P - CL_{organ} * C_{organ}) \quad (2)$$

where tissue-dependent parameters are expressed as V (organ volume), Q (flow rate), CL (organ-level clearance), and K (unbound tissue:plasma partition coefficient); $B:P$ represents whole blood to plasma partition ratio, and C is the drug concentration in the specific compartment.

This global approach has been previously described in our manuscript ⁴².

Given the identified relevant tissues for SARS-CoV-2 infection (nasal, lung, and intestine), a critical aspect of the approach was enabling simulation of compound disposition to those relevant sites-of-action. Generally, the tissues were assumed to be well-mixed; thus, distribution was characterized using a partition coefficient:

$$K_{Tissue:Plasma} = \frac{C_{tissue}}{C_{plasma}} \quad (3)$$

where C_{tissue} is concentration of the compound-of-interest in a particular organ/tissue, and $K_{Tissue:Plasma}$ is the drug/subject-specific partition coefficient in the tissue. The methodology utilized to determine the partition coefficient was derived from the simulation accuracy of the plasma-concentration curve. There are multiple different methodologies that have been utilized in an attempt to predict these partition coefficients accurately, including industry standards such as the Rodgers-Rowland and Schmitt equations, however even these relationships are susceptible to significant variability ⁴³⁻⁴⁵. These models predict compound partitioning as a function of logP (octanol-water partition coefficient), pKa, and plasma protein binding ($f_{u,p}$); however, for specific

groups of compounds they are found to underperform in their predictive capabilities based on the simplifying assumptions used ⁴³⁻⁴⁷⁴³⁻⁴⁷³⁸⁻⁴²³⁸⁻⁴²³⁸⁻⁴²³⁸⁻⁴²³⁸⁻⁴²³⁸⁻⁴²³⁴⁻³⁸³⁷⁻⁴¹³⁷⁻⁴¹³⁷⁻⁴¹³²⁻³⁶³²⁻³⁶³²⁻³⁶[38-42]. We therefore utilized the standard Rodgers-Rowland equation, optimization of the octanol-water partition coefficient, and direct optimization of an average K_p to increase the simulation accuracy for *in vivo* PK. Partition coefficient values were directly optimized for Captopril, Lacidipine, and Verapamil, while octanol-water partition values (logP) were optimized for Spirapril.

The nasal epithelium mechanism for compound distribution involved integration with a permeation across a barrier. Given the location of nasal goblet cells *in vivo*, compound into the epithelium was modeled with assumptions of 1D flux across the barrier derived from Fick's Law. To approximate compound buildup in tissue and effective reduction of the concentration gradient without incorporating semi-infinite sink assumptions, the epithelium thickness was scaled down by a factor of two. To model penetration to the nasal epithelium, a model of small molecule penetration across a permeable barrier was utilized. The developed model was identified and adapted from a combination of approaches utilized for predicting penetration across the barrier:

$$\frac{dC_{Na}}{dt} = Q_{Na} \left(C_a - \frac{BP * C_{Na}}{k_{pNa}} \right) - P_{nasal} * A * \left(\frac{C_{Na}}{K_{pNa}} - C_{nasal_epi} * f_{nasal} \right) \quad (4)$$

$$\frac{dC_{nasal_epi}}{dt} = P_{nasal} A_{surf} \left(\frac{C_{Na}}{K_{pNa}} - C_{nasal_epi} * f_{nasal} \right) \quad (5)$$

where Na refers to the nasal tissue compartment, C_{nasal_epi} the concentration in the nasal epithelium, f_{nasal} the fraction of compound bound to the tissue, P_{nasal} the permeability across the barrier, and A the area across which compound diffusion can occur. Physiological parameters used in the model were aggregated from a diversity of sources ⁴⁸⁻⁵⁰. For the purpose of these simulations, permeability was assumed to be 0.000101 cm/s for all of the compounds studied, and the partition coefficient into the nasal tissue was assumed to be equivalent to that of lung tissue, as reported in Table 2 ⁵¹⁻⁵³

Rapid repurposing workflow

Test Dataset

An internal database largest proprietary curated database consisting of structure-related data for >1M compounds, >3700+ unique *in vivo* plasma concentration-time validation datasets from public and proprietary sources (signed data sharing partnerships) representing ~2000 unique compounds and 83 different subject populations (different species, gender, strain, sub-strain). The dataset was compiled through a combination of automated data scraping and manual data curation, resulting in the development of over 41 coded/automated consistency checks that detect outliers and corrupt data coming from either published literature on FDA compounds or from

ongoing data partnerships. This innovative data curation approach was applied to CCB/ACE data curation, import and validation.

Screening through the internal database of compounds, comprising >2000 small molecules PK parameters & *in vivo* datasets identified five compounds that targeted the pathway-of-interest and had sufficient validation data available for confident & rapid simulation of outcomes. To establish the combinatorial effect of ACEIs/CCBs combinations on COVID-19 infection, an accurate prediction of drug concentration at a site-of-action is required. Figure 2 highlights the approach taken to identify the 5 drugs (3 ACEIs, 2 CCBs) that were candidates for the study as a result of the available *in vivo* clinical data and preclinical PK parameters that were already established. to validate the ability to predict plasma concentration accurately (as the standard metric for PK).

Figure 1: Metrics for candidate compound identification utilizing internal database of small molecule PK. The numbers in blue boxes correspond to the number of compounds identified as acceptable during each stage of curation.

Drug Name	Class	ka	LogP	pKa	fu,p	B:P	Clearance (L/hr/kg)	Kp _{lung}	Kp _{gut}
Lisinopril	ACEI	0.17*	-1.115 ⁵⁴	3.17 (acid), 10.21 (base) ⁵⁴	0.99 ⁵	0.71*	0.072 ⁵	0.57	0.50
Captopril	ACEI	N/A	0.34 ⁵⁵	4.01 (acid), -1.2 (base) ⁵⁶	0.73 ⁵	0.45*	0.72 ⁵	0.15*	0.15*
Spirapril	ACEI	0.53 ⁴	0*	3.62 (acid), 5.2 (base) ⁵⁷	0.314678*	0.74**	0.43 ⁴	0.21*	0.16*
Lacidipine	CCB	1.7843*	5.51 ⁵⁸	19.47 (acid), -6.4 (acid) ⁵⁹	0.05 ⁶⁰	0.70*	1.23 ⁶¹	11.72*	11.72*
Verapamil	CCB	N/A	3.795 ⁶²	9.68 (base) ⁶¹	0.064 ³	0.88 ^{28,62}	0.84 ^{3,5,62}	3.69*	3.69*

Table 3: Classification of identified ACEI, CCB compounds and utilized physicochemical and PK parameter values obtained from literature, default value approximation, or ML optimization.

* Optimized parameter values

**Default value used

Drug Name	Formulation	Experimental Setup	Reference
Lisinopril (Oral)	20 mg oral dose	20 mg of Lisinopril was given orally for 10 consecutive days. 8 subjects in the study.	1
Captopril (IV)	2.78mg, 5.67 mg, 11.4mg IV dose	1 mL of intravenous injection at three different dosage levels was administered to 7 healthy subjects.	5
Spirapril (Oral)	25mg, oral administration	25 mg spirapril p.o. prepared by dissolving 25mg of lyophilized spirapril in 50mL tap water was given to the subjects. 16 subjects.	4
Lacidipine (Oral)	2mg, 4 mg Oral Dose	Single dose of 2mg and 4mg of Lacidipine was administered. The study has a total of 24 subjects (12 male, 12 female)	2
Verapamil (IV)	50 mg IV dose	5 subjects received 5mg (+)verapamil dissolved in 30 ml of saline infused over 5 minutes.	3

Table 4: Background on datasets used for systemic plasma-venous compartment disposition simulation and optimization of missing PK parameters

Statistics and Tools

The statistical methodologies utilized for the data analysis have been detailed in a previous work⁴². Briefly, *in vivo* plasma concentration datasets and associated error bars, when available, were manually digitized from source publications using "WebPlotDigitizer" version 4.2.34. Model development and validation was done using the in-house platform in Python with Cython integration; matplotlib (v2.0.2) and Numpy (v1.14.2) were auxiliary packages used in simulation deployment and analysis. Model validation and analysis of model goodness-of-fit/accuracy was conducted using three quantitative metrics: absolute average fold error (AAFE), average fold-error (AFE), and chi-squared statistic (χ^2) with associated p-value (null hypothesis defined as no difference in predicted vs. experimental measurements). Sensitivity of the model was evaluated using convergence plots generated during optimization of the parameters. Non-compartmental calculations were utilized to compare the accuracy of the simulations to the experimental data; AAFE and AFE were utilized to evaluate the accuracy of the PK outputs AUC_{0-t} , C_{max} , and t_{max} using the general equations:

$$AFE = \text{Average fold error} = 10^{\frac{1}{n} \sum_{i=1}^n \log\left(\frac{\text{predicted}_i}{\text{observed}_i}\right)} \quad (6)$$

$$AAFE = \text{Absolute average fold error} = 10^{\frac{1}{n} \sum_{i=1}^n \left| \log\left(\frac{\text{predicted}_i}{\text{observed}_i}\right) \right|} \quad (7)$$

where n is the total number of compounds used in the analysis and $\text{Predicted}_i/\text{Observed}_i$ correspond to predicted and observed values of PK parameters, respectively. χ^2 was calculated using the relationship:

$$\chi^2 = \frac{1}{n} \sum_{i=1}^n \left(\frac{\text{predicted}_i - \text{observed}_i}{\text{observed}_{error,i}} \right)^2 \quad (8)$$

where observed_{error} is the standard error/deviation in the measurements in the individual experimental data timepoints, obtained by digitizing the error bars from the respective publications. Optimization convergence was driven and measured by AAFE of AUC , C_{max} , and t_{max} . Statistical calculations of AFE, AAFE, and visual plot analysis were done in GraphPad Prism version 8.4.1 (GraphPad Software, San Diego, CA) and Microsoft Excel (2016).

Subjects

Species-specific parameters used in the simulations were adapted from literature sources and included parameters such as organ flow rates, composition, volumes, and protein levels for the physiological compartments in the BIOiSIM model⁶³⁻⁶⁷. Parameters for the model of small molecule diffusion across the nasal tissue and epithelium were obtained from a thorough literature review^{48,49,68,69}.

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Author Contributions

K. Chakravarty, V.G. Antontsev, J. Varshney: Conceptualization, supervision, writing - original draft preparation, writing - review and editing; N. Maharao, H. Hou, A. Jagarapu: data curation, validation, visualization, writing - review and editing; Y. Bunday: software, validation, writing - review and editing. All authors have read and agreed to the submitted version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.