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ORIGINAL RESEARCH

Modelling the Molecular Transportation of Subcutaneously Injected Salubrinal

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Abstract: For the subcutaneous administration of a chemical agent (salubrinal), we constructed a mathematical model of molecule transportation and subsequently evaluated the kinetics of diffusion, convection, and molecular turnover. Salubrinal is a potential therapeutic agent that can reduce cellular damage and death. The understanding of its temporal profiles in local tissue as well as in a whole body is important to develop a proper strategy for its administration. Here, the diffusion and convection kinetics was formulated using partial and ordinary differential equations in one- and three-dimensional (semi-spherical) coordinates. Several key parameters including an injection velocity, a diffusion coefficient, thickness of subcutaneous tissue, and a permeability factor at the tissue-blood boundary were estimated from experimental data in rats. With reference to analytical solutions in a simplified model without convection, numerical solutions revealed that the diffusion coefficient and thickness of subcutaneous tissue determined the timing of the peak concentration in the plasma, and its magnitude was dictated by the permeability factor. Furthermore, the initial velocity, induced by needle injection, elevated an immediate transport of salubrinal at $t < 1$ h. The described analysis with a combination of partial and ordinary differential equations contributes to the prediction of local and systemic effects and the understanding of the transportation mechanism of salubrinal and other agents.

Keywords: drug delivery, salubrinal, subcutaneous injection, diffusion, convection, rats

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Introduction

One of the common forms of drug delivery is subcutaneous administration, in which a therapeutic agent is locally injected into a tissue under a skin. An injected agent diffuses into the subcutaneous tissue near the injection site, and it usually circulates throughout a whole body in a bloodstream. To determine appropriate dosages and frequencies of administration as well as evaluate efficacy of local and systemic therapeutic outcomes, it is important to understand pharmacokinetics by analyzing a mass transportation mechanism and a quantitative profile of concentrations in local tissue and blood (plasma). Using data obtained from animal experiments (rats) with subcutaneous and intravenous injections of salubrinal, we built a mathematical model and characterized the temporal profiles of salubrinal concentrations in the plasma.

Salubrinal is a potential therapeutic agent that can reduce cellular stress such as oxidation and stress to the endoplasmic reticulum, and it has been shown to protect cells from apoptosis and assista metabolic process.[1,2] However, the expected outcomes may depend on its concentration profiles in the plasma as seen in the case of parathyroid hormone (PTH). PTH is often administered as a form of subcutaneous injection for treatments of patients with osteoporosis and related bone diseases.[3] Interestingly, both animal experimentation and clinical data show that a transient increase of PTH yields an anabolic response and stimulates bone formation, while its sustained elevation contrarily induces a catabolic response leading to bone loss.[4,5] Although salubrinal is a synthetic chemical agent and its mechanism of action is largely different from that of PTH,[6,7] it is imperative to quantitatively characterize its delivery efficiency as well as temporal concentrations in the plasma prior to any clinical trials.

Models for pharmacokinetics, simulating the concentration profiles of chemical agents and biomolecules, have been studied in various areas.[8–10] However, most of these models utilize a compartmental description considering only interactions expressed in ordinary differential equations (ODEs). These ODEs serve to predict the general trends of agents injected subcutaneously, but they do not account for time-dependent spatial distributions. Partial differential equations (PDEs) are utilized in some formulations, for instance, in evaluating the effects of administration of insulin.[11] Few models, however, can be analytically solved using a well-characterized set of parameters and thus it is difficult to validate their predicted mechanisms and behaviors.

In this study we combined PDEs and ODEs to account for diffusion, convection, and molecular turnover in the one- and three-dimensional coordinates, and employed analytical solutions in the simplified model without convection to validate numerical solutions of the general model. The model consisted of two modules: a module for diffusion and convection kinetics from the local tissue at the injection site to blood vessels; and the other module for turnover kinetics (degradation of salubrinal) in the bloodstream. In the first module, PDEs were derived to model diffusion and convection of salubrinal. In the second module, these PDEs in the first module were linked to ODEs that included a transfer of salubrinal at the tissue-blood boundary as well as its degradation during blood circulation.

Using experimental data obtained in rats, the parameters in the model such as the injection velocity, the diffusion coefficient, thickness of the subcutaneous tissue, and the permeability factor (effective boundary area ratio of subcutaneous tissue to blood circulation) were estimated. Note that the permeability factor (between 0 and 1) represents the degree of salubrinal to be transmitted at the boundary of subcutaneous tissue to blood vessels. The specific solutions without convection were derived analytically, while the general solutions were obtained numerically by simultaneously solving PDEs and ODEs. Using numerical simulations, we evaluated sensitivities of key parameters and obtained the model-based prediction of the salubrinal profile in the plasma.

Materials and Methods

Experimental determination of the salubrinal concentration in the rat plasma

Experimental data for building the model were collected using Sprague-Dawley female rats (~8 weeks; ~300 g body weight). All procedures performed in this study were approved by the Animal Care and Use Committee. A dosage of 0.5 mg/kg (salubrinal weight/body weight) was administered via intravenous or subcutaneous injection. Data from intravenous injection were used to predict the degradation rate of salubrinal in the plasma. Blood samples were collected at various time points at 1–22 h after salubrinal injection.

The concentrations of salubrinal in the plasma were determined from three animals at each time point by mass spectrometry at Wolfe Laboratories Inc. (MA).

Diffusion and molecular turnover in the one-dimensional model

In the one-dimensional coordinate, the concentration of salubrinal in the subcutaneous tissue in the vicinity of the injection site was derived:

$$\frac{\partial C_t}{\partial t} = D\frac{\partial^2 C_t}{\partial x^2} \quad (1)$$

In which C_t = concentration of salubrinal in the tissue, and D = diffusion coefficient. This equation can be solved analytically as one-dimensional Gaussian diffusion bounded on one side by the skin at $x = 0$ (Fig. 1):[12]

$$C_t(x,t) = \frac{M}{\sqrt{\pi \mathrm{Dt}}} e^{-\frac{x^2}{4Dt}} \quad (2)$$

where M = injected amount of salubrinal. Assuming that salubrinal is eliminated from the plasma at a constant rate k_d, the rate of change of salubrinal in the plasma is expressed:

$$\frac{dC_b}{dt} = \frac{k_s M}{2V_b\sqrt{\pi Dt^3}} x_b e^{-\frac{x_b^2}{4Dt}} - k_d C_b \quad (3)$$

In which C_b = concentration of salubrinal in the plasma, k_s = permeability factor (effective boundary area ratio between 0 and 1), V_b = blood volume, and x_b = thickness of the subcutaneous tissue. Note that V_b (ml) was estimated as $V_b = 0.06w + 0.77$, in which w = body weight (g).[13]

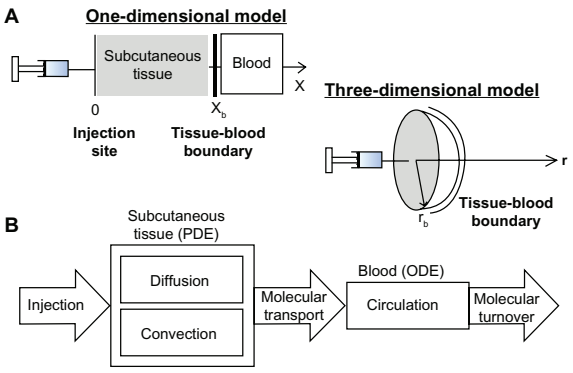

Figure 1. Schematic illustration of the described model. **A**) Configuration of the subcutaneous tissue and blood. **B**) Linkage of the PDE module to the ODE module.

Diffusion and molecular turnover in the three-dimensional model

In the three-dimensional coordinate (semi-spherical domain; Fig. 1), the salubrinal concentration in the subcutaneous tissue (C_t) is modeled:

$$\frac{\partial C_t}{\partial t} = \frac{D}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial C_t}{\partial r}\right) \quad (4)$$

In which r = radial coordinate. The concentrations in the subcutaneous tissue and the plasma (C_b) are expressed:

$$C_t(r,t) = \frac{M}{4(\pi Dt)^{\frac{3}{2}}} e^{-\frac{r^2}{4Dt}} \quad (5)$$

$$\frac{dC_b}{dt} = \frac{k_s M}{2V_b\sqrt{\pi \mathrm{D}^3 \mathrm{t}^5}} r_b^3 e^{-\frac{r_b^2}{4Dt}} - k_d C_b \quad (6)$$

where r_b = thickness of the subcutaneous tissue.

Convection induced by the needle injection of salubrinal

To evaluate the effects of the initial velocity induced by needle injection, a term for convection was included in the subcutaneous tissue. In the one-dimensional coordinate, the PDE becomes:

$$\frac{\partial C_t}{\partial t} + v\frac{\partial C_t}{\partial x} = D\frac{\partial^2 C_t}{\partial x^2} \quad (7)$$

where the injection velocity was modeled as $v = v_0 e^{-\frac{t}{T}}$ with v_0 = initial velocity, and T = time constant (3 min).

Estimation of the key parameters

Table 1 lists the representative values for the key parameters employed in this study. The values for the amount of injection, and the blood volume were

Table 1. Representative parameter values employed in this study.

Symbol	Definition	Value	Unit
M	Amount of injection	150	μg
V_b	Blood volume	18.8	ml
k_d	Degradation rate in plasma	0.59	1/h
k_s	Permeability factor (effective boundary area ratio)	2.5×10^{-2}*; 5.0×10^{-3}**	–
D	Diffusion coefficient	1.8×10^{-6}	m^2/h
x_b or r_b	Tissue thickness	3×10^{-3}	m

Notes: *The value for the one-dimensional model; **The value for the three-dimensional model.

chosen independently from the mass spectrometry data. The degradation rate, k_d, was derived from the concentration profile of salubrinal in response to intravenous injection. The time constant, $(1/k_d)$, was 1.2 h. The four parameters (initial velocity, diffusion coefficient, tissue thickness, and permeability factor) were estimated from data for subcutaneous injection. An approximate range of initial velocity was predicted from the injection volume and the needle size, and adjusted using numerical results. The diffusion coefficient was estimated from the known value for dexamethasone in the subcutaneous tissue.[14] Note that the molecular masses of dexamethasone and salubrinal are 392 Da and 480 Da, respectively.

The permeability factor (k_s), which determined the fraction of salubrinal that entered from the subcutaneous tissue into the bloodstream, was linked to the density of blood vessels at the tissue-blood boundary. This factor was estimated from experimental data and numerical simulations. The rates of change in salubrinal concentrations in Eqs. (3) and (5) were modeled to be proportional to $D^{1/2}$ and $D^{3/2}$ in the one- and three-dimensional models, respectively. To adjust mean-square diffusional distances, we employed the same value of D in both models with unique k_s values for each of the two models. Thus, the value of k_s for the three-dimensional model was set to approximately one fifth $(\sim 1/3\sqrt{3})$ of the one-dimensional model. Note that the above factor of 3 was an idealized difference of mean-square diffusion distance in these one- and three-dimensional models without considering convection.

Numerical simulations

The schematic diagram illustrating the computational procedure is depicted (Fig. 1). The described models were numerically evaluated using MATLAB (version 7.8.0). The PDEs without a term of convection were analytically obtained. The PDEs including convection were solved numerically using a discrete difference method implemented by the *pdepe* function. The solutions were then incorporated into ODEs, which were integrated using the function *ode45*, an implementation of the Runge-Kutta method.[15]

Results

Effects of diffusion and molecular turnover in the plasma

We first examined the pharmacokinetics model for diffusion and molecular turnover without including the convection term. In the one-dimensional model, the selected parameters were k_s (permeability factor) = 2.5×10^{-2}; D (diffusion coefficient) = 1.8×10^{-6} m^2/h; and x_b (thickness of the subcutaneous tissue) = 3×10^{-3} m (Fig. 2). These parameters were chosen to match the predicted peak concentration value equal to the highest data point from experimentation. Note that the value of $k_s < 1$ indicates that the fraction of $(1 - k_s)$ was not transported into blood circulation and absorbed locally in the subcutaneous tissue and interstitial fluid. Using the same values for the diffusion coefficient and thickness of the subcutaneous tissue ($r_b = x_b$), the concentration profile in the three-dimensional model was also predicted (Fig. 2). The value of $k_s = 5 \times 10^{-3}$ in the three-dimensional model was chosen to make the peak concentration value identical to that in the

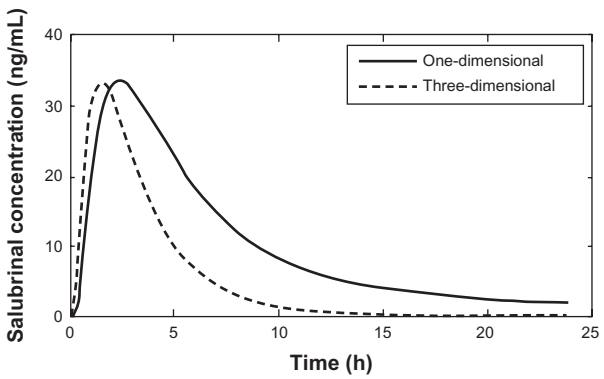

Figure 2. Comparison of one- and three-dimensional models without convection.

one-dimensional model. Although the overall concentration profiles presented the same transient behavior, the results indicated that a transfer of salubrinal in the three-dimensional model than the one-dimensional model.

Sensitivity analysis of the parameters

Using both one-and three-dimensional models, a sensitivity analysis was conducted to evaluate dependence of the temporal profile of salubrinal concentrations in the plasma on the diffusion coefficient and tissue thickness (Fig. 3). In the figure, the predictions with three different values for each of the parameters are illustrated. First, increasing the diffusion coefficient enhanced the transport of salubrinal into the blood stream, generating a higher and quicker peak value in the plasma. Second, thickening the subcutaneous tissue decreased the entry of salubrinal to the plasma, causing a slower transient response with a lower and longer peak (Fig. 3B). These features were observed both in the one- and three-dimensional models.

Effects of convection and injection velocity

In addition to diffusion and molecular turnover, we examined the effects of convection by introducing the initial velocity at the time of salubrinal injection into the subcutaneous tissue. In consistent with the experimental procedure, we modeled the initial velocity as an exponential function with a time constant of 3 min

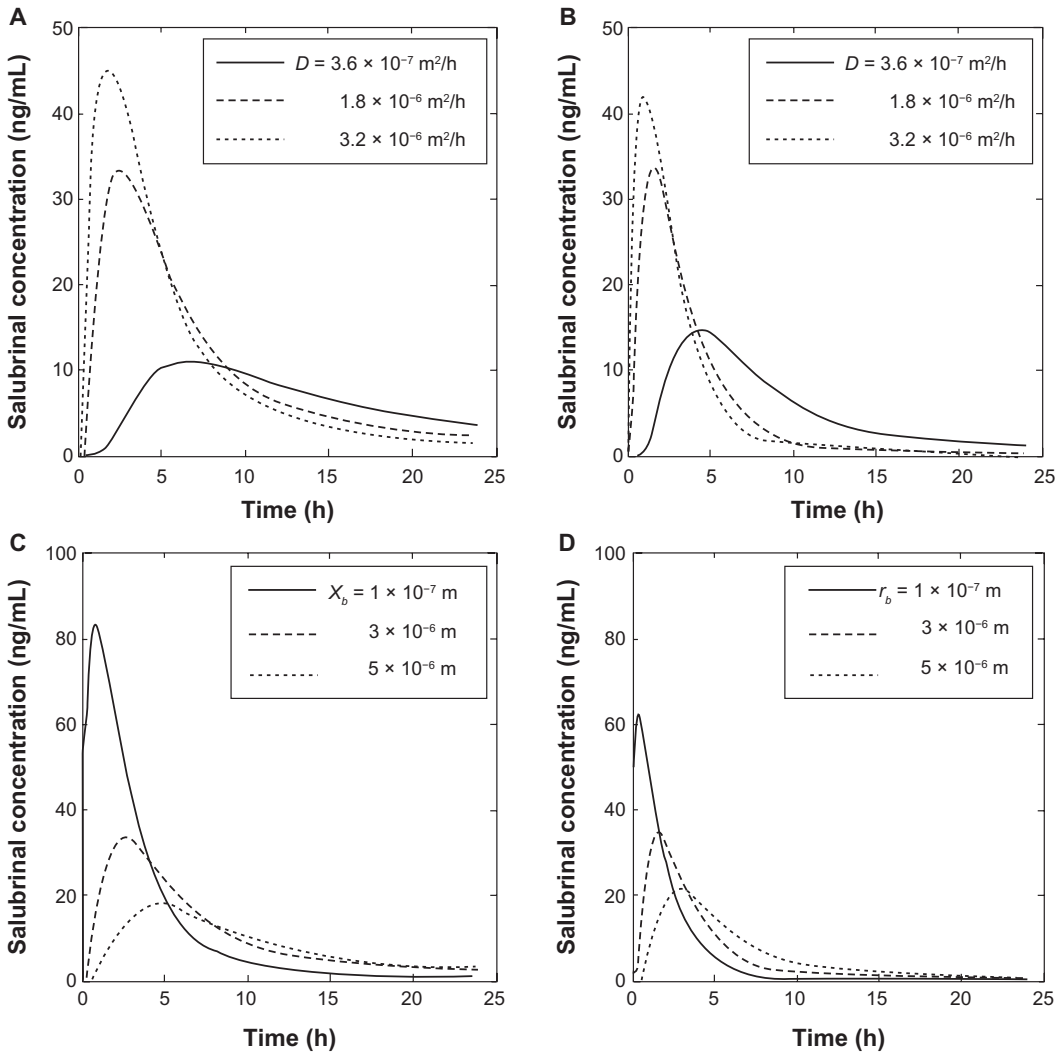

Figure 3. Parameter sensitivities. **A**) Dependence on *D* (diffusion coefficient) in the one-dimensional model. **B**) Dependence on *D* in the three-dimensional model. **C**) Dependence on x_b (tissue thickness) in the one-dimensional model. **D**) Dependence on r_b (tissue thickness) in the three-dimensional model.

that corresponded to a typical duration of injection. The predicted response with the initial velocity of 2×10^{-2} m/s is depicted (Fig. 4). Compared to the model without convection, the time required to reach the peak concentration value was shortened and this prediction became closer to the experimental data. The effects of convection were further analyzed by varying the initial velocity (Fig. 5). The result showed that the larger injection velocity was, the quicker rise of the peak salubrinal concentration was generated with the higher peak value in the plasma.

Evaluation of experimental data

Using the described model, the concentration profile, obtained by experimentation, was fitted by the numerical solutions for the one-and three-dimensional model (Fig. 6). In this figure, the experimental data points are shown by the dots. The parameters were selected to offer a minimum square-sum error at 6 given data points. The convection term reduced the modeling error at $t = 1$ h, but it did not significantly reduce the modeling error at $t = 16$–22 h. The three-dimensional model gave smaller error at a later stage than the one-dimensional model.

Discussion

We conducted analytical and numerical simulations to evaluate experimental data for the salubrinal concentrations in the plasma in response to subcutaneous administration. The model combined the PDE-derived diffusion and convection process to the ODE-based turnover of salubrinal in the bloodstream

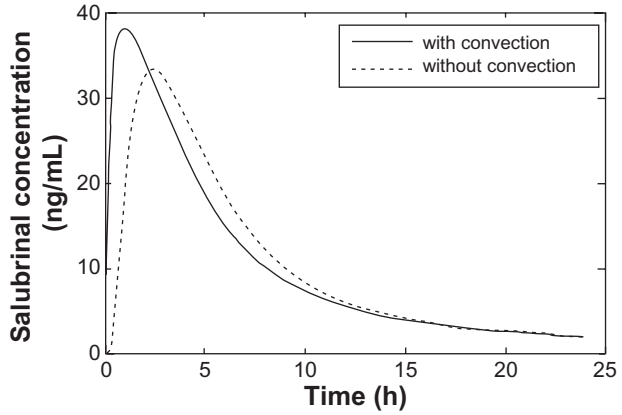

Figure 4. Comparison of the salubrinal concentration profiles in the plasma using the models with and without convection. The solid and dotted curves are the models with and without convection, respectively.

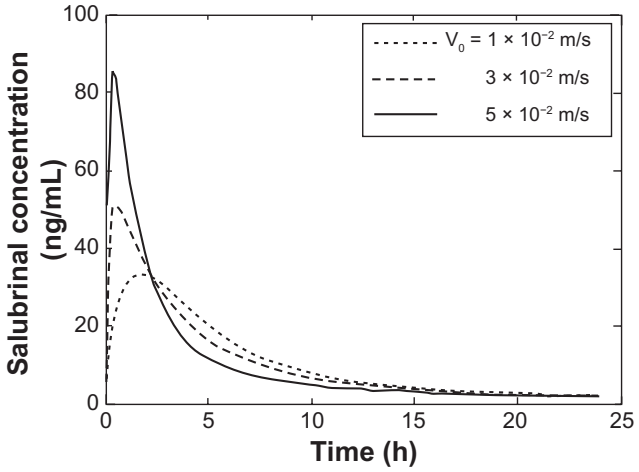

Figure 5. Dependence on the initial velocity. Three values, chosen for the initial velocity, were 1, 3, and 5 cm/s.

using one- and three-dimensional (semi-spherical) coordinates. With reference to analytical solutions of the simplified model without convection, the model-based analysis herein allowed us to estimate the key parameters in pharmacokinetics and predicted the temporal profiles as well as the delivery efficiency in local tissues and a whole body.

The diffusion-based model without convection in the PDE module was able to characterize the processes of diffusion, transportation at the tissue-blood boundary, and molecular turnover during blood circulation. In both the one- and three-dimensional models, there was a quick increase to the peak concentration followed by an exponential decay of the salubrinal concentrations in the plasma within a day. Using the same set of parameter values, the three-dimensional model exhibiteda slightly faster transient response. Namely, applying the same diffusion coefficient and tissue thickness to the models, the three-dimensional version ascended more quickly to the peak and descended both earlier and more steeply than the one-dimensional version. This model-based result captured the principal differences in these two models, since the three-dimensional coordinate provided the spatial domain with two more degrees of freedom. The permeability factor for the three-dimensional model was close to the predicted value of one fifth of that for the one-dimensional model. The results also indicated that 0.5%–2.5% of the total amount of salubrinal was transported to the blood stream and the rest was absorbed in local tissue and interstitial fluid.

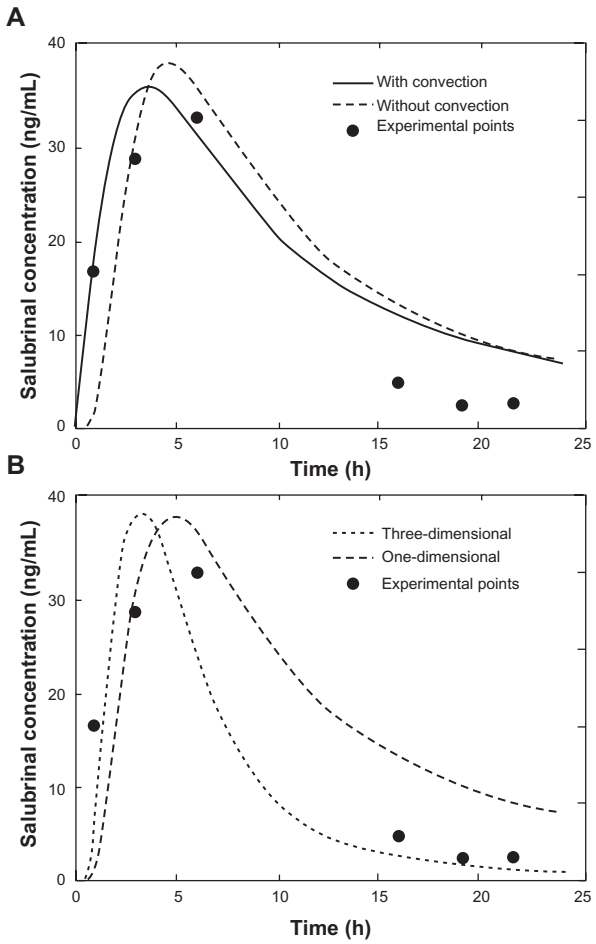

Figure 6. Comparison of the model-predicted concentration profiles to the experimental data. The initial velocity and tissue thickness were set to 2 cm/sec and 5.2 mm, respectively. The permeability factor was chosen to be 0.057 (one-dimensional) and 0.0095 (three-dimensional). **A**) Salubrinal concentration profiles in one-dimensional model with and without convection. **B**) Salubrinal concentration profiles in one- and three-dimensional models.

As expected from a biophysical viewpoint, the diffusion coefficient and the tissue thickness affected the temporal profile of the salubrinal concentration in the opposite way. An increase in the diffusion coefficient accelerated the diffusive transportation and elevated the peak of the salubrinal concentration, whereas an increase in the tissue thickness decreased both diffusive efficiency and the peak value. A larger diffusion coefficient implies that molecular agent scan diffuse more quickly, while a larger tissue thickness indicates that there is a greater distance through which salubrinal has to travel to reach the tissue-blood boundary. We assumed that the diffusion coefficient was constant, but it may differ depending on specific tissue locations and diffusive directions. Furthermore, we used 3 mm as tissue thickness for experimental data collected from rats but this thickness may need to be modified when clinical data are employed.

The convection term improved the model at the initial time point. In the absence of convection, we observed a slower rise of the salubrinal concentration than the experimental data points, particularly at t = 1 h. The inclusion of convection made this deviation smaller, since salubrinal transportation was significantly elevated by convection and this elevation rate was faster than the rate of degradation in the blood. Since the injection itself apparently introduced a non-zero transfer velocity, we modeled this convection effect using an exponentially decaying velocity profile during 3-min administration duration. It was not possible to reduce the deviation of salubrinal at the initial phase only by adjusting the diffusion coefficient or the tissue thickness without significantly offsetting the timing and the level of the peak concentrations. However, the inclusion of convection tended to predict higher levels of salubrinal in the plasma at t = 16–22 h. Taken together, the results in the current study are consistent to the prediction that inclusion of the convection accelerates transport of salubrinal to the blood stream. To model an initial increase in the salubrinal concentrations, inclusion of the convection term improves predictions. To estimate the concentration profile at a later degradation phase, the three-dimensional version is apparently superior to the one-dimensional version although the consideration of other parameters may improve the one-dimensional model.

Salubrinal was originally identified as a selective inhibitor of a phosphatase specific to eukaryotic initiation factor 2 alpha (eIF2α), whose phosphorylation level is regulated by various cellular stresses including oxidation, nutrient deprivation, radiation, and the stress to the endoplasmic reticulum.[16–18] Administration of salubrinal was reported to reduce cellular stress and cellular death.[1] and chemical analogues to salubrinal were also examined to enhance efficacy to protect cells from apoptosis.[19] We previously applied control theories to derive an administration sequence of salubrinal for treatment of metabolic diseases.[20] The described models in this paper should contribute to development of dosages, frequencies and durations of administration of salubrinal and other chemical agents.

Conclusion

We described the novel mathematical model for estimating the plasma concentration of salubrinal in rats in response to subcutaneous injection. The molecular transportation by diffusion and convection was formulated using PDEs, while the molecular turnover in blood circulation was described by ODEs. The analytical solutions without convection were employed to validate the numerical procedure, and the experimental data were used to select the key parameters such as the diffusion coefficient, thickness of the subcutaneous tissue, the permeability factor, and the injection velocity. The results supported the notion that subcutaneous administration of salubrinal could induce both local and global circulations.

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Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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