Agent-based model of therapeutic intervention following exposure to botulinum neurotoxin

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Abstract

Neurotoxins produced by the bacterium *Clostridium botulinum* are highly toxic in very low doses and can be used as biological threat agents. Symptoms begin from 30 minutes to 6 days and include nerve palsies and blurred vision leading to flaccid paralysis and respiratory failure. Full recovery begins after 3 months and can take up to a year. Botulinum neurotoxin (BoNT) is taken up into nerve terminals at the neuromuscular junction (NMJ) and blocks transmission of nerve signals, resulting in paralysis. While much data exists on the in vivo targets of these neurotoxins and their interactions, the mechanisms involved in intoxication, therapeutic intervention and natural recoverability are not completely understood. We are developing an in silico, agent-based platform to test hypotheses related to treatment and recovery following exposure to BoNT. This platform leverages high performance computing (HPC) to explore parameter space and study the differences between deterministic and stochastic models. The foundation of this platform is a quantitative kinetic model simulating the steps of BoNT-induced muscle paralysis. A comparison of model predictions to experimental results indicates that the model requires further refinement. A few points under refinement include addition of multiple muscle fibers and nerve sprouting. We will discuss the current state of the model and our efforts to account for experimental data.

1 Introduction

BoNT binds to specific receptors on nerve terminals at the neuromuscular junction (NMJ) and is taken up into the cell by endocytosis [13, 14]. Once inside an endosome, BoNT escapes into the cytoplasm and binds to and cleaves components of the SNARE complex.

This complex is required for exocytosis of acetylcholine into the synaptic cleft; by preventing formation of the SNARE complex, BoNT blocks release of acetylcholine and transmission of nerve signals, resulting in paralysis. Using known rate constants, a quantitative kinetic model, described below, simulating these steps and the natural recovery in a single nerve terminal has been developed [9]. Kinetic models provide a useful tool for checking assumptions and testing hypotheses and have been used to study such phenomena as immune synapse formation [12], polarization of neutrophils during chemotaxis [11], and mitogen-activated protein kinase cascades [3].

An in vitro assay for studying the effects of botulinum toxin and potential therapeutics is the mouse hemidiaphragm preparation [1]. This assay measures the tension that develops in a mouse hemidiaphragm suspended in solution and stimulated via the attached phrenic nerve. BoNT and therapeutic compounds are added to the solution to measure the resulting effects. Experimental data arising from this assay shows a decay in the tension leading to paralysis on addition of BoNT [1]. Addition of antibodies delays the onset of paralysis by sequestering a portion of the neurotoxin. Different antibodies delay paralysis by differing amounts. To date, the kinetic model is unable to predict experimental results using different concentrations of BoNT and different antibodies without making significant adjustments to the rate parameters. This suggests that the model may be incorrect. The hemidiaphragm consists of a number of muscle fibers each connected to the phrenic nerve via a nerve terminal. Our initial efforts will focus on including multiple muscle fibers to more realistically model the assay.

Muscle paralysis leads to release of cytokine signals and neurotrophic substances that cause sprouting to occur from affected nerve terminals [7]. Once developed, these sprouts are similar to the original nerve terminal and act to functionally compensate for the BoNT-affected NMJ. When the BoNT is eliminated and the original nerve terminal regains its activity, the sprouts retract. The pathways that lead to production of neurotrophic substances and sprouts are currently unclear. These sprouts likely play an important role in

therapeutic intervention and recovery. They may also contribute to development of resistance to BoNT over time. Clinical data in which BoNT was used to treat neurological symptoms shows that resistance develops after several cycles of treatment and recovery [6]. One of our goals is to develop an in silico platform to test hypotheses about the mechanisms behind this phenomenon.

There are several potential strategies for therapeutic intervention related to each step of the BoNT neurotoxic pathway [10]. Inhibitors can be developed that (1) compete with BoNT for binding to the receptor on the nerve terminal membrane, (2) prevent BoNT release from endosomes, or (3) block BoNT binding and cleavage of SNARE components in the cytoplasm. Questions arise as to the optimal strategy and design parameters as well as the dosing regimens that take advantage of the dynamics of the system. Another aim of this research is to test hypotheses about these potential treatment options.

The presence of multiple muscle fibers and nerve terminals that have similar functionality and interact through signaling events lends itself to simulation using agent-based modeling (ABM). ABM defines a collection of autonomous agents and rules for their interactions and is useful for capturing complex behavior patterns that would be difficult to study with traditional differential equations [4]. ABMs have been used to study the dynamics of the innate immune system response [2], pathogen transmission [8], and multi-cellular tumor spheroid growth [5]. Our model will consist of agents representing nerve terminals. Each agent will contain a copy of the kinetic model and will interact with its surroundings via chemical messengers.

High performance computers will be used to simulate the model. A single node can represent a nerve terminal agent with a collection of nodes representing the entire mouse hemidiaphragm. The application of multiple runs using different rate parameters will enable a rapid exploration of parameter space. Model variability will also be examined.

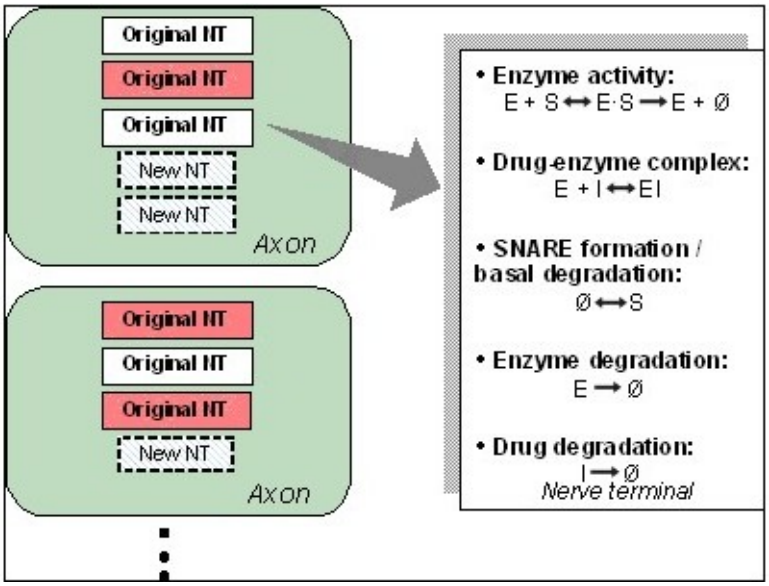

Figure 1: An agent-based model of BoNT neurotoxicity.

2 Model

As an illustration, our method of accounting for nerve sprouting uses a combination of an agent-based modeling approach with kinetic rate equations. Figure 1 shows a brief overview of this approach. The environment that contains the agent nerve terminals is an abstraction of the extracellular space in a neuromuscular junction and has dynamic concentrations of BoNT (B), antibody (A), and an unspecified neurotrophic substance (NTS). The agent nerve terminals contain internal states describing species concentrations based on kinetic rate equations described below.

Interaction between the agent nerve terminals occurs through NTS. Production of NTS can be abstracted in the model in several ways. For example, it can be related to BoNT concentration or the decrease in SNARE concentration. When NTS reaches a critical value within a nerve terminal, sprouting of a new, child nerve terminal occurs. Formation of the new nerve terminal is then assumed to take a certain period of time, and retraction occurs based on the activity of the parent nerve terminal.

The kinetic equations describing the species concentrations are defined as follows: BoNT

(B) in the extracellular space is given by

$$\frac{d(B)}{dt} = -k_b(B)(R) + k_u(R_B) - k_{dB}(B) \tag{1}$$

where k_{dB} is the elimination rate constant, and k_b and k_u describe binding and unbinding to receptors (R), respectively. Bound receptors (R_B), given by

$$\frac{d(R_B)}{dt} = k_b(B)(R) - k_u(R_B) - k_i(R_B) \tag{2}$$

are internalized with rate constant k_i to form BoNT-containing endosomes (R_{Bi}). BoNT-containing endosomes are destroyed when BoNT escapes with rate constant k_{esc} as follows:

$$\frac{d(R_{Bi})}{dt} = k_i(R_B) - k_{esc}(R_{Bi}). \tag{3}$$

Once inside the cytoplasm of the nerve terminal, BoNT takes on an enzymatic form (E), given by

$$\frac{d(E)}{dt} = k_{esc}(R_{Bi}) - k_f(E)(S) + k_r(E_S) - k_{dE}(E) \tag{4}$$

where k_f and k_r describe binding and unbinding to the SNARE (S) to form BoNT-SNARE complexes (E_S) and k_{dE} is a pseudo-first order rate constant describing elimination of BoNT by enzymatic or other means. SNARE (S), given by

$$\frac{d(S)}{dt} = k_{pS} - k_f(E)(S) + k_r(E_S) - k_{dS}(S) \tag{5}$$

is synthesized at the rate k_{pS} and has a basal elimination rate described by k_{dS}. The concentration of BoNT-SNARE complexes is then given by

$$\frac{d(E_S)}{dt} = k_f(E)(S) - k_r(E_S) - k_{f2}(E_S) \tag{6}$$

where k_{f2} describes the cleavage of S to form product P which is eliminated. Antibody reversibly binds BoNT in the extracellular space and leads to its removal from the system. The concentration of antibody (A) in the extracellular space is given by

$$\frac{d(A)}{dt} = -k_a(A)(B) + k_{-a}(A_B) \tag{7}$$

where k_a is the binding rate constant and k_{-a} is the unbinding rate constant.

For other therapeutic compounds introduced into the system, additional equations describing their concentrations depend on the particular targets and mechanisms of action. Additional terms may be necessary to account for diffusion of NTS and other factors between nerve terminals.

3 Future Work

The first priority in this project is to refine the kinetic model to account for experimental results from the mouse hemidiaphragm assay. This effort will involve agent-based modeling to include multiple muscle fibers and nerve terminals. High performance computers will be used to explore parameter space before and after this extension. With a validated model, different therapeutic options and recovery and resistance via nerve sprouting will be examined.

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