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Toxin effect on protein biosynthesis in eukaryotic cells: A simple kinetic model

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ABSTRACT

A model for toxin inhibition of protein synthesis inside eukaryotic cells is presented. Mitigation of this effect by introduction of an antibody is also studied. Antibody and toxin (ricin) initially are delivered outside the cell. The model describes toxin internalization from the extracellular into the intracellular domain, its transport to the endoplasmic reticulum (ER) and the cleavage inside the ER into the RTA and RTB chains, the release of RTA into the cytosol, inactivation (depurination) of ribosomes, and the effect on translation. The model consists of a set of ODEs which are solved numerically. Numerical results are illustrated by figures and discussed.

1. Introduction

Ricin, a natural product of the castor bean (*Ricinus communis*), is highly toxic to mammalian cells. It has been used for oncological cancer treatment, but it also has the potential to be used as a weaponised biological agent because of its stability and ease of production [1,2]. Ricin poisoning can cause severe tissue damage, inflammatory reactions and can result in death [3]. In spite of intensive biomedical research no treatment is available for ricin poisoning. Some promising results have been shown recently using an immunotherapeutic approach, i.e. application of high affinity antibodies to neutralise the effects of ricin [4,5].

The development and production of new antibodies is an expensive process that usually includes extensive experimental studies. In this context, the retrospective evaluation of antibodies with continuous experimental refinement of its binding parameters aiming at selection of the best candidate may become very time and resource consuming. In order to reduce this experimental burden a simple modelling framework derived from mass-action kinetics has been recently proposed [6–8].

The process of toxin entry into a cell, associated with bio-chemical reactions and their toxic effects, is well documented. This includes receptor–toxin binding, toxin internalization, intracellular transport to the endoplasmic reticulum (ER) and inhibition of protein synthesis [9–12]. The modelling framework reported in [6–8] ignores some fine details of this complex and multi-stage process and aims to capture them by means of a set of coupled mass action kinetics equations.

The aim of the present study is to extend the framework reported in [6–8] and to model the last stage of the toxicological effect of ricin: inhibition of protein synthesis in the cell.

Protein synthesis is a complex biochemical process [13,14]. Models of protein synthesis have been analysed in numerous publications and it is still a topic of intensive research, see [15–32].

Protein synthesis in eukaryotic cells begins with the transcription of a messenger RNA (mRNA) by RNA polymerase from a given gene (DNA) in the nucleus of cells. This mRNA then is transported into the cytosol where a cellular ribosome binds to it to make a new protein from the specific amino acids carried by aminoacylated ('charged') transfer RNAs (tRNAs). Process of incorporation (translation) of tRNA carried amino acids into a polypeptide chain (protein) is realised according to the sequence of the mRNA and can be divided into three stages: (i) initiation, in which ribosome joins the mRNA and locates the first (initiation) codon, (ii) elongation, in which the ribosome moves in a codon by codon stepwise manner along the mRNA, using tRNA carried molecules to incorporate the appropriate encoded amino acid at each codon, and (iii) termination, in which the last (stop) codon is recognised by a protein release factor to direct release of the completed polypeptide from the ribosome.

The effect of ricin on protein synthesis is a result of the ability of ricin to 'deactivate' (damage) a ribosome and exclude it from the pool of ribosomes available for protein synthesis (by depurinating the α-sarcin/ricin loop of the large rRNA [33,34]). This allows us to

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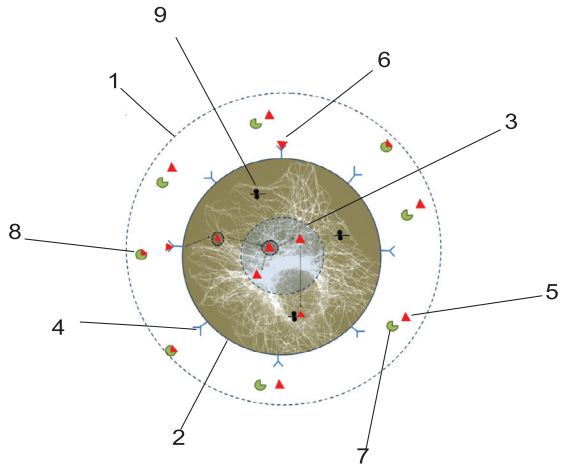

Fig. 1. Ricin pathway to ribosome: 1 – spherical surface of the external compartment (extracellular domain Ω_e), 2 – cell membrane (external boundary of intracellular domain Ω_i, 3 – spherical envelope of ER, 4 – receptor, 5 – toxin, 6 – toxin–receptor complex, 7 – antibody, 8 – toxin-antibody complex, 9 – ribosome.

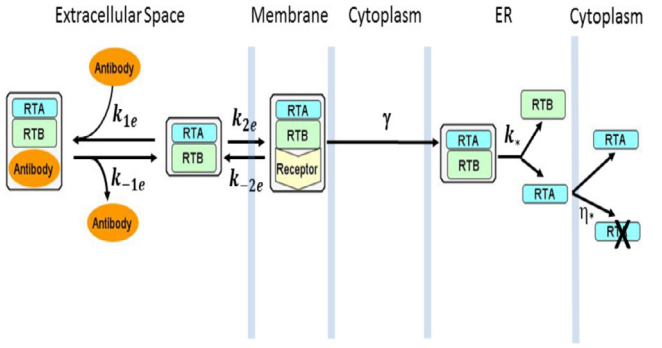

Fig. 2. Ricin chemical transformation during pathway to ribosome: values of rate constants are given in Table 1, notations for chemical species are given in the main text.

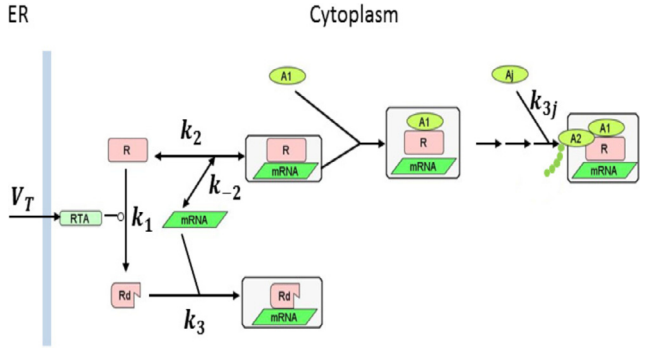

Fig. 3. Chemical bindings of ricin in the ribosome pool in the cell cytoplasm: values of rate constants are given in Table 1, notations for chemical species are given in the main text.

propose a simple phenomenological model of the process (mass-action kinetics) that captures the effect of ricin as a 'supply-demand' constraint imposed on the ribosome pool. For the sake of simplicity we disregard the processes of aminoacylation of tRNA (i.e., activation of tRNA during which a specific amino acid is joined to tRNA), mRNA transcription and details of its translation by a ribosome (i.e., reading and linking together of two neighbouring amino acids into a polypeptide chain) and neglect post-translational modifications of the synthesised protein.

By employing this mass-kinetic model we also demonstrate how introduction of an antibody can mitigate inhibition of protein synthesis. More specifically, we consider a model in which the toxin (ricin) and an antibody are initially delivered outside the cell and study their effect on cell protein synthesis in these settings.

The paper is organised as follows. In Section 2 we present the model. In Section 3 we discuss numerical results and summary in Section 4 concludes the paper.

2. The model

Our model aims to capture two main bio-chemical processes associated with the toxicological effect of ricin: ricin transport to the ribosome and the effect of ricin on the ability of the ribosome to synthesise protein. The geometrical settings of the system are depicted in Figs. 1 and 3. The schemas of chemical reactions describing transformations in bio-chemical processes are presented in Figs. 2 and 3.

To make predictions that may be validated in a laboratory setting, the model is based on isolated, cultured cells and other factors involved in *in vivo* intoxication, such as clearance rate, non-uniform distribution throughout the tissue, etc., are not considered. The concentrations of reacting species used in the model are typical of those used in laboratory studies and the number of molecules involved is sufficiently large, so that the system is usually well described by the deterministic equations for the mean values of fluctuating concentrations. We note that this assumption may not be always valid during the early stages of ricin transport into the cell when the number of internalised toxin molecules is small (i.e. stochastic fluctuations caused by a finite number of molecules in the system may become significant [35–37]

We begin with consideration of the process of ricin transport to ribosome. In order to model a geometrically extended system with a single-cell settings we assume a spatial periodicity of the system and impose a special boundary condition at some radius of the single-cell 'compartment' $\rho_e \gg \rho_m$ (external domain), where ρ_m and ρ_e are radii of the cell membrane and external sphere, respectively. The cell is modelled as a sphere surrounded by a permeable membrane (for details see [7,8]) and ER is modelled as an enclosed concentric sphere of a smaller radius $\rho_n \ll \rho_m$ with a partially absorbing boundary. To shorten notations we use Ω_e for extracellular domain ($\rho_e \gg \rho_m$) and Ω_i for intracellular space (i.e. the domain between the ER envelope and the cell membrane, $\rho_n \ll \rho_m$), see Fig. 1.

Let toxin (ricin) and antibody be initially delivered in the extracellular domain (i.e. in Ω_e). Then according to [7,8]) the concentration of species in Ω_e is governed by equations

$$\begin{cases} \dot{u}_{Te} = -k_{1e}u_{Te}u_{Ae} + k_{-1e}u_{Ce} \\ \qquad\quad - k_{3e}r_0(k_{2e}(1-\theta)u_{Te} - k_{-2e}\theta), & u_{Te}(0) = u^0_{Te}, \\ \dot{u}_{Ae} = -k_{1e}u_{Te}u_{Ae} + k_{-1e}u_{Ce}, & u_{Ae}(0) = u^0_{Ae}, \\ \dot{u}_{Ce} = k_{1e}u_{Te}u_{Ae} - k_{-1e}u_{Ce}, & u_{Ce}(0) = 0, \\ \dot{\theta} = k_{2e}(1-\theta)u_{Te} - k_{-2e}\theta - k_e\theta, & \theta(0) = 0 \end{cases} \tag{1}$$

where $u_{T_e}, u_{A_e}, u_{C_e}$, and θ are concentration of toxin (T_e), antibody (A), toxin-antibody complex (C), and fraction of toxin-bound receptors, respectively, $u^0_{T_e}$ and $u^0_{A_e}$ are initial concentrations of the toxin and antibody, k_{1e} and k_{-1e} are association and dissociation toxin-antibody rate constants, k_{2e} and k_{-2e} are association and dissociation toxin-antibody rate constants of toxin–receptor binding. Parameter $r_0 =$ const is the concentration of receptors on the cell surface, so that $r_0\theta$ is the concentration of toxin-bound receptors [7,8].

From a solution of this system we can determine the flux of the internalised toxin across the cell membrane into the intracellular domain Ω_i (this flux is equal to $k_e r_0\theta$). Inside Ω_i toxin moves toward the ER driven by the mechanism of retrograde transport and eventually crosses the ER envelope [34]. By applying the mass-balance