Mathematical Model Reveals Increased Protease Following Inhibition Due To Cannibalistic Regulation

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Introduction: Proteolytic remodeling of elastin and collagen in the arterial wall contribute to atherosclerotic initiation and progress. Cysteine cathepsins are powerful elastases and collagenases, and have been implicated under biochemical and biomechanical stimuli that lead to plaque progression. Cathepsins K, L, and S are upregulated in endothelial cells subject to disturbed blood flow, where plaques preferentially develop. Pharmaceutical companies have developed cathepsin inhibitors and a number have entered clinical trials, with some ending early due to off target effects.[1] Cathepsin expression and activity are regulated by endogenous protein inhibitors as well as proteolytic interactive networks that are not completely understood. Through in vitro studies, we have recently shown that cathepsin levels are subject to cathepsin-on-cathepsin cleavage, or cathepsin cannibalism. This has unexpected implications for protease cross-reactivity with pharmaceutical inhibitors, and may explain the observed off target effects. To better understand the complex system of proteolytic regulation, and improve the design of treatments aimed at decreasing cathepsin mediated arterial remodeling, we have constructed and validated a computational model of cathepsins K and S activity towards elastin, collagen, and each other to simulate the effects on matrix degradation following treatment with protease inhibitors of cells during atherosclerosis.

Materials and Methods: The cathepsin-substrate-inhibitor model was formulated as a system of ODEs and was implemented using in Matlab. Parameters were estimated from literature using a combination of a genetic algorithm and the lsqnonlin Matlab optimization function. Time course enzymatic data was obtained through experiments performed in the lab by incubating recombinant cathepsins K, L, and S under differing combinations with fluorogenic gelatin and elastin substrates that could be quantified in a spectrophotometer.

Results and Discussion: At steady state, [catK] < [catS] due to catS degradation of catK (Figure 1). Dosing with cathepsin inhibitor leads to rapid binding of free catS and catK, but the catS catK complex unbinds more slowly. Once the inhibitor is depleted, free cathepsin, catK and catS rebound and catS binds catK again. Following inhibition, MatK initially overshoots then drops to its steady state due to catS degradation (Figure 1). This overshoot persists for several hours and could cause elevated cathepsin K levels following dosage with small molecule inhibitors. This elevation could accelerate arterial remodeling due to increased degradation of elastin and collagen in the arterial wall. These simulations indicate that cannibalism by catS can successfully regulate concentrations of catK, but this regulation is not instantaneous, and can result in transiently increased catK levels following perturbation with cathepsin inhibitors.

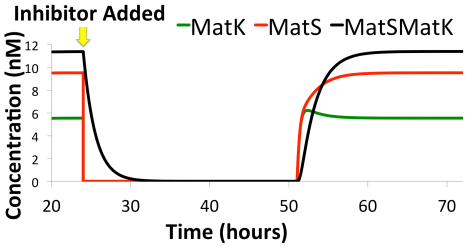

Figure 1. Simulation of cathepsin inhibitor dosage at 24 hours. Free Cathepsins K and S are bound by inhibitor, but over time inhibitor is depleted and cathepsin concentrations recover with cathepsin K overshooting its steady state.

Conclusions: These simulations suggest a nonspecific cathepsin inhibitor could cause transient increases in cathepsin K concentrations because of cathepsin-on-cathepsin regulation. We further hypothesize cathepsin specific inhibitors could result in increased proteolytic activity caused by disrupting this regulatory network. The development of a computational regulatory model that includes kinetics of cathepsin activation and cannibalistic interactions will allow us to better predict how perturbations to the steady-state proteolytic network will affect both protease and substrate levels underlying the arterial wall remodeling during disease, and inform proper dosing and use of selective cathepsin inhibitors to prevent the unexpected side effects that have been seen previously in cathepsin inhibitor studies.

References: [1] D. Brömme, Expert Opin Investig Drugs. 2009 May; 18(5): 585–600.