Dynamic Bioluminescence Imaging: Development of a Physiological Pharmacokinetic Model of Tumor Metabolism

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Bioluminescent imaging (BLI) has proven to be a valuable tool for the study of cellular biology and therapeutic response in a wide array of tumor types. Several BLI analytical approaches have been developed to assess tumor function and growth, all with the primary assumption that substrate concentrations saturate the luciferase enzyme. Recent work suggests that when D-luciferin is administered over the range from 75-600mg/kg, target tissue concentrations of D-luciferin are well below the Km of luciferase for the reaction, and, that the pharmacokinetics of D-luciferin significantly impact observed emission rates. To address the concentration and PK concerns, we developed a three compartment physiologically based pharmacokinetic (PhPK) model for D-luciferin including oxidation by luciferase via Michaelis-Menten kinetics. The model was applied to dynamically acquired BLI in NOD/SCID mice with ectopic luciferase-transfected SF767 tumors. The current PhPK model estimates tumor volume, tumor substrate metabolism ($\bar{M}$), tumor blood flow (Vb) and substrate extraction from the blood (Er). Studies were conducted using intraperitoneal, subcutaneous and intravenous routes of administration of 150 mg/kg of D-luciferin, where dynamic BLI was conducted weekly for four weeks. The D-luciferin concentration in tumor tissue, determined immediately after the last imaging session, was found to be approximately 8-fold below the reported Km for the reaction across all routes of administration, supporting the need for a PhPK modeling approach for analyzing BLI data. The model-predicted tumor volumes increased over time and were highly correlated with caliper-measured tumor volumes ($y=1.984x$, $R2=0.980$, $p<0.0001$). Tumor D-luciferin metabolism was found to increase exponentially over the 4 weeks, while blood flow decreased over this same interval, a finding which is consistent with the interpretation of a Warburg effect. When tumor $\bar{M}$ was compared with the traditional measures of peak emission (Cmax) and area under the curve (AUC), it was found that metabolism increased exponentially with increases in either Cmax ($y=92.7\exp(8E-11x)$, $R2= 0.997$) or AUC ($y=86.4\exp(5E-14x)$, $R2= 0.989$), suggesting that Cmax and AUC may substantially underestimate the magnitude of tumor metabolism. The present PhPK model of D-luciferin distribution and metabolism overcomes limitations in the Cmax and AUC approaches caused by incorrect substrate: enzyme concentration assumptions, and thus provides a more reliable estimate of tumor burden, growth, and therapeutic response.