Review of: "The Fundamental Problem With Enzyme Inhibition Theory"

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Potential competing interests: No potential competing interests to declare.

Manuscript Review for Qeios

Title: *The Fundamental Problem with Enzyme Inhibition Theory*

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Reviewer: Ross L. Stein

In this manuscript, Dr. Walsh derives new equations for the analysis of enzyme inhibition. Unfortunately, he does not demonstrate how his new approach is superior to traditional approaches.

First, let me say that I'm all in favor of new and innovative ways to treat data. However, if a new method is going to be valuable, it must solve a problem that current methods cannot solve. Dr. Walsh does not make clear what problems cannot be solved by traditional methods of enzyme kinetics.

He does hint at this problem in the first sentence of the Conclusions section: "The use of the traditional inhibition term represents a failure to distinguish between the chemical equilibrium for enzyme-inhibitor binding and the effect the inhibitor produces when it binds to the enzyme". The "effect" to which he refers is presumably how the bound inhibitor might induce a conformational change that either changes the catalytic efficiency of the enzyme or a conformational change that causes even tighter binding of the inhibitor. The former is exemplified by allosteric inhibitors, which has been treated quite well by traditional ways of analyzing kinetic data *(1)*, and the latter occurs with reversible, time-dependent inhibitors (a.k.a., slow-binding inhibitors), and again has been treated well by traditional methods *(2)*.

To demonstrate that his new methods of enzymatic data analysis are needed, Dr. Ryan should discuss several examples from the literature where traditional methods fail, but his method succeeds. I assume he must have many examples to share, since I can't imagine he would develop this new method with no actual problems to solve.

Also, it would be beneficial if he were to discuss the traditional methods he opposes. Of course, there is not a single 'traditional' approach to the analysis of enzyme inhibition data, but what they have in common is that they are mechanism-driven. The starting point for analysis is an examination of the raw data, perhaps the dependence of steady-state velocity on substrate concentration at several fixed concentrations of inhibitor. From this examination, the investigator will be able

to deduce a potential mechanism. Based on this presumed mechanism, an equation is derived and the data fit to the equation. If the fit is poor, the investigator revises the mechanism, and the data is then fit to the new equation.

An example of this method is on pp. 104 – 105 of my kinetics textbook*(3)*, where I analyze published data for the inhibition of the ATPase activity of Eg5. The data I analyze is the dependence of velocity on ATP concentration at several concentrations of the inhibitor. While inspection of the data clearly indicates substrate inhibition, the investigator did not take this into account and incorrectly fit the data to a simple model for competitive inhibition. A much better fit is obtained when the data is fit to an equation that takes substrate inhibition into account.

In the Conclusion section, Dr. Ryan discusses how many of the databases that tabulate enzymatic data assume simple Michaelis-Menten. I am not sure if this is true or not, but even if it is true, this is either due to the databases not being careful about how they report data or investigators using the incorrect model to analyze data. Non-Michaelian enzyme kinetics has been recognized for over half a century and has been dealt with successfully with the use of standard mechanism-based kinetic equations.

Finally, as an aside, I believe that controversial statements need to be supported. In the first sentence of the abstract, Dr. Ryan states: “The failure of modern enzyme inhibition drug theory stems from the derivation of the inhibition equations.” In what way has “modern enzyme inhibition drug theory” failed? In fact, I’d have to say “enzyme inhibition drug theory” has been extremely successful. As of 2005, 317 of the 1,278 approved drugs are enzyme inhibitors *(4)*. In the final section of the paper, he tells us that “the field of enzyme kinetics has expanded in artificial complexity.” Again, a statement like this should be further explained (frankly, I’m not sure what he means) and must be supported by citing relevant scientific literature. If Dr. Ryan cannot explain this and support his opinion with concrete examples from the scientific literature, such statements should be removed from the paper.

[1] Stein, R. L. (2011) Allosteric Modulation of Enzyme Activity, In*Kinetics of Enzyme Action - Essential Principles for Drug Hunters*, John Wiley & Sons, Hoboken, N.J.

[2] Stein, R. L. (2011) Tight-Binding, Slow-Binding and Irreversible Inhibitors, In*Kinetics of Enzyme Action - Essential Principles for Drug Hunters*, p 6, John Wiley & Sons, Hoboken, NJ.

[3] Stein, R. L. (2011) *Kinetics of Enzyme Action - Essential Principles for Drug Hunters*, John Wiley & Sons, Hoboken, N.J.

[4] Robertson, J. G. (**2005**) Mechanistic Basis of Enzyme-Target Drugs,*Biochemistry44*, 5561-5571.