Comments on “Drug combination studies of curcumin and genistein against rhodesain of *Trypanosoma brucei rhodesiense*”

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ABSTRACT

Recently, it was suggested that curcumin is an irreversible inhibitor of rhodesain, a cathepsin L-like cysteine protease found in the lysosome of the protozoan parasite *Trypanosoma brucei*. However, dilution of rhodesain incubated with curcumin with curcumin free buffer resulted in an immediate recovery of enzymatic activity. This finding clearly demonstrates that curcumin is in fact a reversible inhibitor of rhodesain.

KEYWORDS

Rhodesain; curcumin; reversible inhibitor

Dear Editor,

I would like to comment on the article “Drug combination studies of curcumin and genistein against rhodesain of *Trypanosoma brucei rhodesiense*” by Ettari et al. (2018). In their article, the authors claim that curcumin and genistein are irreversible inhibitors of rhodesain (the major lysosomal cysteine protease of *T. brucei*) and that both compounds in combination act synergistically in inactivating the enzyme. However, it is not possible that two irreversible inhibitors can interact synergistically in inhibiting the activity of an enzyme. By definition, irreversible inhibitors chemically modify their target enzyme by reacting with the active-site amino acid residue. Accordingly, two compounds irreversibly inhibiting an enzyme cannot influence each other on their binding of the catalytic amino acid residue. This is only possible if one of the compounds binds the enzyme at a different site. Thus, the binding of one compound could increase the affinity for the other compound and vice versa resulting in a synergetic effect of the combination of the two compounds.

The evidence provided by the authors to support that curcumin and genistein are irreversible inhibitors of rhodesain are unconvincing. The irreversible inhibition of the compounds were based (i) on a time-dependent loss of enzyme activity and (ii) on the lack of recovery of enzymatic activity after dilution. However, the time-dependent inhibition was only marginal and the enzyme was never completely inhibited, even at higher inhibitor concentrations. Likewise, the dilution factor in the dilution experiment was minimal (only 1:2), so that the final inhibitor concentration was still very high. In the case of genistein, the high IC_{50} value (500 µM) observed for the inhibition of rhodesain activity is quite unusual for an irreversible inhibitor. Generally, irreversible inhibitors of *T. brucei* cysteine proteases have IC_{50} values below 1 µM and those with IC_{50} values > 100 µM are usually considered inactive (Steverding et al. 20112).

The lack of convincing evidence whether curcumin and genistein are irreversible inhibitors of rhodesain prompted me to perform own experiments. Based on available reagents, I carried out dilution experiments with curcumin (Figure 1). Incubation of

rhodesain with 10 µM curcumin resulted in an enzymatic rate of 2.74 RFU/min. When the reaction mixture was diluted 1:4 with measuring buffer containing 10 µM curcumin, the enzymatic rate dropped to 0.74 RFU/min, which was 27% of the activity before dilution and thus in agreement with an one in four dilution of the amount of enzyme (the expected activity would have been 25%). In contrast, when the reaction mixture was diluted 1:4 with measuring buffer lacking curcumin, the enzymatic rate increased to 12.82 RFU/min, which was 4.68 and 17.32 times higher compared with the starting mixture and after dilution in the presence of 10 µM curcumin, respectively. Were curcumin an irreversible inhibitor of rhodesain, no difference in the enzymatic rate between the dilution in the presence and absence of 10 µM curcumin would be expected. As the enzymatic rate after dilution in curcumin free measuring buffer increased substantially (despite a 4-fold reduction in enzyme concentration), thus it can be concluded that curcumin is a reversible inhibitor of rhodesain and not an irreversible one.

The lesson, here, is: in order to determine the mode of action of inhibitors, one needs to carry out the right experiments and to interpret the obtained data correctly. The best way to do this would be to perform rigorous kinetic enzyme analysis.

Disclosure statement

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Figure Legends

Figure 1. Reversibility of curcumin inhibition on rhodesain. Rhodesain (67 ng) was incubated in measuring buffer (100 mM citrate, pH 5.0, 2 mM DTT and 10 µM benzyloxycarbonyl-phenylalanyl-arginyl-7-amido-4-methyl coumarin (Z-FR-AMC) as fluorogenic substrate) in the presence of 10 µM curcumin in a final volume of 2 ml at room temperature. The release of 7-amino-4-methylcoumarin (AMC) was recorded with a BIORAD VersaFluor fluorometer using excitation and emission wavelengths of 360 nm and 460 nm every min (triangles). After 20 min incubation, the reaction mixture was diluted 1:4 with measuring buffer either containing 10 µM curcumin (squares) or the equivalent amount of DMSO (circles), and release of AMC was continued to be recorded. Note that the drop in RFU after dilution with measuring buffer containing 10 µM curcumin is due to the dilution of released AMC. Note that the increase in RFU after dilution with measuring buffer lacking curcumin is due to the fact that curcumin shifts RFU into negative values. Therefore, by diluting curcumin containing buffer with curcumin free buffer, RFU increases. RFU, relative fluorescence unit.

105 **Figure 1**

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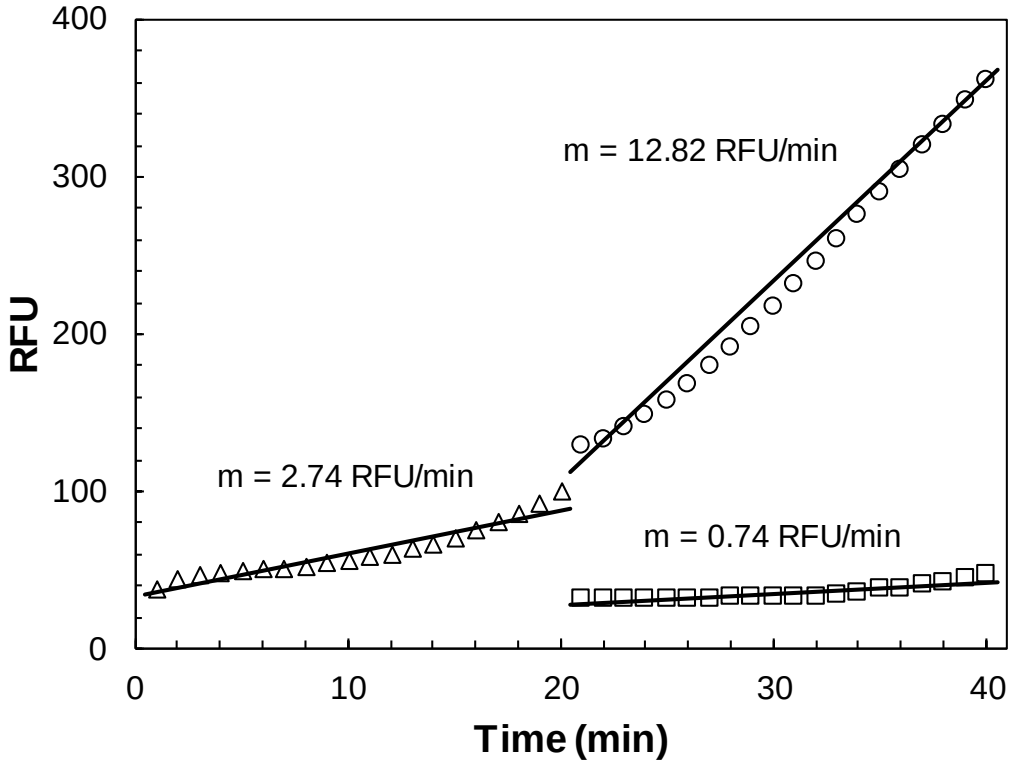

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