Inhibition of Pathogenic Homologs of Glycogen Synthase Kinase (GSK)

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Introduction

- Glycogen synthase kinase (GSK) is implicated in several non-infectious diseases [1] (**Fig. 1**), and inhibitor and GSK inhibitors have entered phase 2 clinical trials for some of these [2].
- GSK homologs in parasites have been suggested as drug targets for infectious disease, if there is selectivity over human GSK [3,4].
- New treatments for diseases like aspergillosis (caused by fungi *Aspergillus* spp.) and leishmaniasis (caused by excavate *Leishmania* spp.)
- *Aspergillus fumigatus* GSK (AfGSK) and *Leishmania donovani* GSK (LdGSK) are potential new drug targets, previously unreported in the literature.

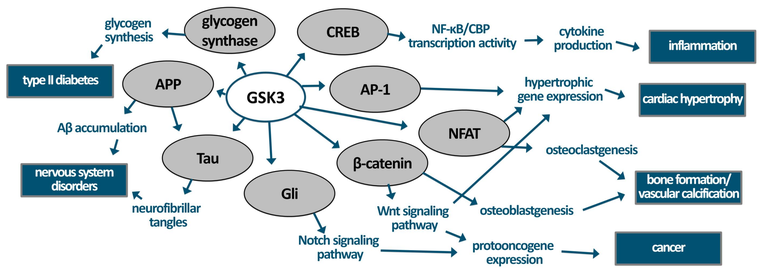

Figure 1. Implications of GSK in non-infectious disease. Adapted from reference [1].

Methods

- AfGSK and LdGSK were cloned, expressed, and purified by Biozilla (Dallas TX) [3,5].
- GSM was used as a substrate for pathogenic GSKs [6]. ADP-Glo (Promega, Madison WI) was used for kinase assays in 384-well plate format by manufacturer's instructions, and ADP produced by GSK reaction (**Fig. 2**) was calculated by light produced [3].
- Plots were generated with GraphPad Prism (Boston MA).

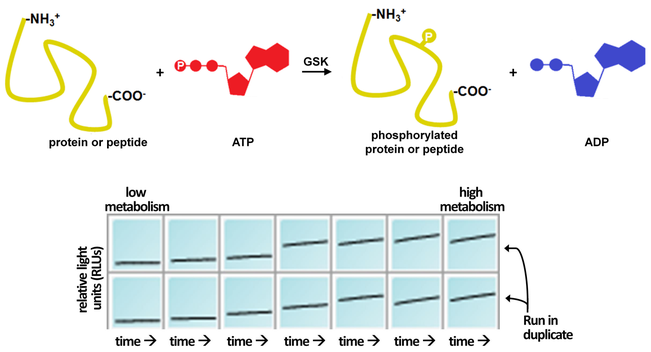

Figure 2. Reaction scheme of GSK and instrument output in a typical experiment.

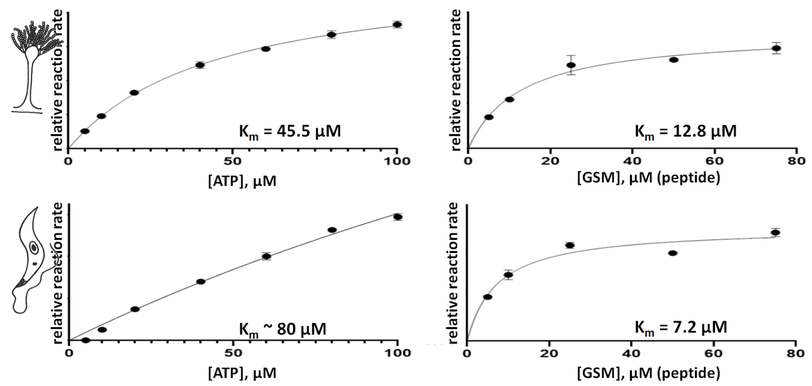

Figure 3. Enzymatic characterization of AfGSK (top) and LdGSK (bottom). Michaelis-Menten constants are shown for ATP and GSM peptide. For LdGSK, Km of ATP is estimated. Plots show representative experiments, run in duplicate, and error bars are SEM.

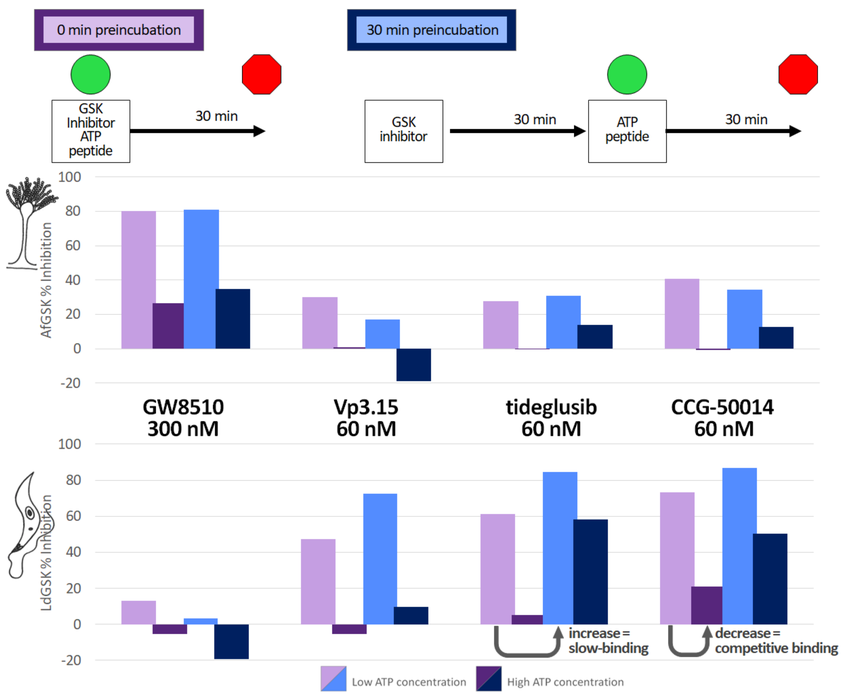

Figure 4. Inhibition by inhibitors discovered for human proteins. Light violet and blue represent experiments run at ATP approximating 0.5 Km (22.5 µM for AfGSK and 40 µM for LdGSK, and dark violet and blue represent concentrations of ATP approximating 5 x Km (45 µM and 80 µM, respectively). All experiments were run with 10 µM GSM and 30 nM AfGSK or LdGSK. For violet experiments, enzyme-catalyzed reactions were treated concurrently with inhibitors; for blue experiments, GSK was treated with inhibitor in a 30-minute preincubation before substrates were added. A loss of inhibition from low ATP to high ATP concentrations indicates competition of the inhibitor for ATP. A gain of inhibition from no preincubation to 30-minute preincubation indicates slow-binding behavior of the inhibitor. Vp3.15 and tideglusib were designed as inhibitors of human GSK3, with reported IC50s of the human homolog of 900 nM [7] and 105 nM [8]. GW8510 was designed as a cyclin-dependent kinase 2 (CDK2) inhibitor and CCG-50014 is an inhibitor of human regulator of G-protein signaling 4 (RGS4).

Results

- AfGSK and LdGSK displayed Michaelis-Menten kinetics (**Fig. 3**), with K_m values of 45.5 µM (AfGSK) and around 80 µM (LdGSK) for ATP and of 12.8 µM and 7.2 µM for the peptide GSM.
- Two human GSK inhibitors, Vp3.15 [7] and tideglusib [4,8] showed inhibition against the pathogen GSKs, with higher activity against LdGSK (Fig. 4)
- GW8510 [4], an inhibitor of human cyclin-dependent kinase 2 (CDK2) had more activity against AfGSK than LdGSK (**Fig. 4**).
- CCG-50014, an inhibitor of human regulator of G-protein signaling 4 (RGS4) had more activity against LdGSK (**Fig. 4**).
- All four compounds exhibited competition for ATP. Vp3.15, tideglusib, and CCG-50014 exhibited slow-binding behavior against LdGSK with no noted effect with AfGSK (**Fig.4**)

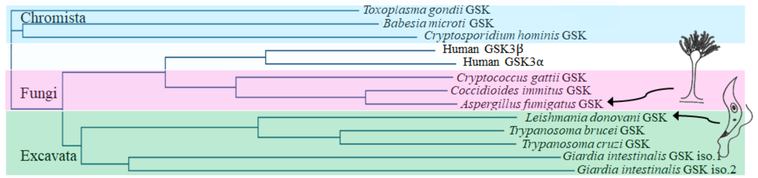

Figure 5. Phylogram of AfGSK, LdGSK, human GSKs, and other homologs from other eukaryotic pathogens.

Future Directions

- Confirm activity and slow-binding activity of these compounds.
- Based on chemical structural similarities of tideglusib and CCG-50014, screen other similar molecules for structure-activity relationships.
- Test other homologs of GSK from other eukaryotic pathogens (**Fig. 5**).
- Counterscreen inhibitors that have not been tested against human GSK3. AfGSK and LdGSK share approximately 63% and 46% identity with human GSKs and about 45% identity with each other.

References

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