Mechanical assays uncover diverse Eg5 inhibitor mechanisms

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The mitotic kinesin Eg5/KSP contributes to spindle formation, regulates neuronal growth, and enhances microtubule (MT) polymerization. Drugs such as monastrol and ispinesib, which act as ATP-uncompetitive inhibitors, generally modulate loop L5 or its proximal regions, stabilizing the nucleotide and trapping the motor in the weak-binding (ADP or ADP-Pi) state. Several ATP-competitive inhibitors that generate a strong-binding apo-state have also been reported. Here, we used mixed-motor gliding assays to reveal the influence of small molecule inhibitors on the mechanical performance of Eg5. Microtubules moved by populations of kinesin-1 and Eg5 motors move near the slow Eg5 gliding speed due to this motors' "braking" ability. In this assay, monastrol and ispinesib result in faster gliding speeds, consistent with Eg5 being in a weak-binding state, whereas the inhibitor BRD9876 slowed gliding in all cases, consistent with it being inhibited in a strong-binding state. In single-molecule experiments, the lack of motor diffusion in the presence of BRD9876 further supports this conclusion. Single-molecule and bulk biochemical assays reveal that BRD9876 serves as an ATP- and ADP-competitive inhibitor of Eg5, suggesting that BRD9876 interferes with the nucleotide-binding and creates an apo-state motor that binds strongly to the MT. The MT-uncompetitive behavior shows that drug binding requires the conformational change associated with MT binding by the motor. This drug-induced strong-binding state provides an alternate strategy of strong-binding state rather than a weak-binding state for inhibiting bipolar spindle formation. Additionally, this strong-binding motor may serve as an effective "brake" to slow down the neuronal growth, or serve as a stabilizer to reduce MT catastrophe.