A Phenotypic Screening Assay of Mouse Egg Activation Identifies Novel Inhibitors Targeting WEE2 for Non-Hormonal Contraceptive Applications

Hoi Chang Lee[1], Jeffrey Pea[1], Alisha F. Ramos[1], Prianka H. Hashim[1], Hidenori Takahashi[2], Christian Andersen[2], Felicia Gray[2], Shelby Ellery[2], Francesca E. Duncan[1]

1. Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL
2. Schrödinger LLC, New York, NY, Portland, OR and San Diego, CA

WEE2 is an oocyte-specific kinase that mediates the resumption of meiosis from the metaphase II arrest at fertilization and is essential for the egg-to-embryo transition. Thus, developing compounds that selectively inhibit WEE2 without affecting kinases such as WEE1 and MYT1 represents a promising strategy for non-hormonal contraceptive development. We established a phenotypic assay for mouse egg activation to assess potential WEE2 inhibitors using a closed time-lapse imaging system (EmbryoScope+™) whereby we tracked second polar body extrusion (PB), pronuclear (PN) formation, and two-cell cleavage. Strontium chloride ($SrCl_2$), a known inducer of calcium transients, was utilized to trigger synchronous egg activation. Mouse oocytes were treated for 2-hour with $SrCl_2$ followed by 24-hour culture. In the absence of selective commercially available WEE2 inhibitors, we used Adavosertib during egg activation as a reference compound to validate the platform. Adavosertib potently inhibits both WEE1(Ki: 0.6 nM) and WEE2 (Ki: 10 nM), but it lacks WEE2 specificity. 24-hours of Adavosertib treatment (500 nM) markedly inhibited egg activation, with significant reductions in developmental progression (2nd PB extrusion: 87.5%, PN-formation: 3%, 2-cell cleavage: 3%) compared to controls (2nd PB extrusion: 100%, PN-formation: 100%, 2-cell cleavage: 94%). Additionally, just a brief 2-hour Adavosertib exposure during egg activation was sufficient to disrupt development. We then tested a novel series of highly selective Wee2 inhibitors developed by Schrodinger (B-1: WEE2 KI <1.5 nM, B-2 WEE2 Ki = 9 nM) and an inactive enantiomer (B-3: WEE2 KI > 650 nM). These compounds are not selective for WEE1 (Ki > 10,000 nM). WEE2 functions to phosphorylate the kinase CDC2 to drive egg activation. Therefore, we assessed target engagement by evaluating expression of pCDC2 and CDC2 by increasing WEE2 activity in activated eggs treated with 10 μM B-1 and B-2 via Western blotting. pCDC2 expression increased in eggs following egg activation, but exposure to B-1 and B-2 inhibited this activity. Total CDC2 and WEE2 levels were similar across groups. Thus, B-1 and B-2 effectively inhibit WEE2 activity. When testing these inhibitors using our phenotypic egg activation assay (in 24-hour culture with compound), 2nd PB extrusion was similar across groups (Control: 100%, B-1: 100%, B-2: 100%, B-3: 100%), but PN-formation (Control: 100%, B-1: 0%, B-2: 0%, B-3: 81%) and cleavage (Control: 100%, B-1: 0%, B-2: 0%, B-3: 59%) were reduced compared to controls. Additionally, these phenotypes were dose dependent. Overall, these findings

demonstrate promising novel WEE2 inhibitors, and studies are ongoing to examine their safety and efficacy in vivo as novel female contraceptive agents.

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