Structure-based identification of novel KN1.1 inhibitors: a stratified target for KCNT1-related epilepsies
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1. Background
• Missense, heterozygous, gain-of-function mutations of KN1.1, a Na+-activated K+ channel encoded by KCNT1 are associated with several, pharmacoresistant epilepsies in children that are accompanied by psychomotor and intellectual disabilities. Malignant migrating partial seizures in infancy (MPSI) and autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE) are two examples, but a number of other early-onset epileptic encephalopathies (EEGE) have also been identified [1,2,3]. There is a wide phenotypic spectrum associated with KCNT1 mutations, and no specific inhibitors acting on the channel, making both prediction of disease outcome and treatment difficult [4]. The channel is widely distributed in the nervous system, and is thought to be involved in generation of the slow afterhyperpolarization following a single action potential or trains of action potential firing [5].

• There are currently three known inhibitors of the channel: the antihypertensive drugs quinidine, bepridil, and cilnidip [6,7]. All are non-selective and inhibit cardiac K+ and/or Na+ channels. Clinically, quinidine has had poor results, likely due to its non-selectivity and lack of potency. It is therefore important to develop a novel, selective inhibitor of KN1.1.

The structure of the chicken KN1.1 channel was recently resolved using cryo-EM [8]. We hypothesised that this structure could be used to identify novel inhibitors in silico by docking a library of compounds into the pore-forming region of the channel. We have identified six novel compounds, all of which are more potent inhibitors of KN1.1 than quinidine in vitro.

2. Methods
• Molecular docking was conducted using the cryo-EM structure of chicken KN1.1, using SwisDock and GLIDE. Firstly, quinidine and bepridil were docked into the pore domain to identify potential binding sites, which were then used for high-throughput screening using a Chembridge library of 100,000 commercially-available compounds. 17 high-scoring compounds were selected, ordered, and dissolved in DMEM (10 mM).

• HEK293-M5R cells were transiently transfected with wild-type (WT) or mutant human KN1.1 constructs, or HERG.

• Currents were recorded by whole-cell patch clamp electrophysiology, using both voltage pulse and ramp protocols. Physiological solutions were used, with 10 mM Na+ included in the pipette (extracellular) solution for KN1.1 recordings. Compound stock solutions were diluted with extracellular solution, and perfused onto cells serially in increasing concentrations. Concentration-response curves were fitted using an Hill function, from which IC50 values could be derived.

• Cell viability was assessed using a WST-1 assay of non-transfected (NT) HEK293 cells cultured in 96-well plates and measuring absorbance at 450 nm. Cell viability was determined using the equation: % cell viability = (Acell/Acontrol) experiment well/Acell/Acontrol control well) x 100.

3. Results I

• Representative currents from wild-type patches expressing WT or mutant KN1.1 with increasing concentrations of quinidine as indicated. B Inhibition-inhibition plots for wild-type and mutant KN1.1 channels in response to 3µM-1mM quinidine. IC50 for WT, 124.99 ± 34.52 µM (n=5); F348S, 73.68 ± 24.49 µM (n=5); F348S, 1.23 ± 0.13 µM (n=4); M354S, 99.22 ± 49.61 (n=5); M354S, 247.16 ± 19.96 µM (n=5); Y796H, 38.00 ± 12.99 µM (n=5). C Comparison of the three compounds for wild-type and mutant KN1.1 channels in response to 3µM-100µM bepridil. IC50 for WT, 6.36 ± 2.12 µM (n=5); F348S, 35.01 ± 11.00 µM (n=5); F348S, 23.43 ± 5.17 µM (n=5).

• Functional evaluation of top-scoring molecules from in silico docking. A WT KN1.1 conductance, relative to baseline, in the presence of 10 µM test compound; with those that were active (right of dashed line) counter-tested with F348S KN1.1 pore mutant (p<0.05, **p<0.005). B Representative traces and C mean (±SEM, n=5) concentration-inhibition plots for active inhibitors. D Summary table with mean potencies of inhibitors, including quinidine and bepridil.

4. Conclusions
• A phenylalanine residue, F346, in the pore-forming region of KN1.1 is important for binding of the channel by quinidine and bepridil.

• Epilepsy-causing mutation Y796H increases quinidine sensitivity of the channel.

• The structure of the chicken KN1.1 channel resolved by cryo-EM was successfully used to identify novel inhibitors of the channel using computer-aided methods.

• Reduced efficacy of the six compounds with F346S suggests they are specifically inhibiting the pore-forming region of KN1.1.

• These are potential tool compounds or novel starting points for developing KN1.1-specific inhibitors, though some may have toxic effects.

5. References/acknowledgements

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