Evaluating the Arrhythmic Potential of Vanoxerine in Human iPSC Derived Cardiomyocytes on a Multiwell MEA.

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Abstract

Vanoxerine was initially developed as a dopamine transporter antagonist for use in the treatment of cocaine addiction but has more recently advanced in clinical trials for the treatment of arrhythmia caused by atrial fibrillation. It's development as a cocaine addiction treatment was halted because it was found to be a potent hERG blocker. It was determined to be a multichannel inhibitor which also blocked Cav1.2 and Nav1.5 which were believed to counteract the hERG effects. This multichannel block was also the reason that it was proposed to be effective in terminating atrial fibrillation. In a phase III study, although vanoxerine was found to be effective in 69% of patients for treatment of atrial fibrillation, the trial was halted due to the development of torsades de pointes in 11.5% of patients. Identification of these liabilities much earlier in the drug development process would save significant money and would also protect patients from these liabilities. To this end, a consortium was established to evaluate a battery of in vitro assays called the Comprehensive In Vitro Proarrhythmia Assay (CIPA). The CIPA initiative is a 3 tiered approach: Test activity in selected cardiac ion channels, predict the effects of these ion channels with in silico computer modeling, and then test the compounds in human stem cell derived cardiomyocytes on an electrophysiology platform such as an MEA. This compound was tested for its ion channel effects and was determined to be safe using the in silico models which suggests that additional assays may be required to identify these liabilities. Here we show that with addition of the third assay, the MEA assay with iPSC derived cardiomyocytes, a liability could have been flagged. Cells were treated with concentrations of 0.01, 0.032, 0.1, 0.32, and 1μM, within the range of the total Cmax. The compound extended the Field Potential Duration (FPD) at all concentrations tested to varying degrees with rounding and flattening of the T-wave above 0.1μM suggesting hERG inhibition. The Na amplitude and slope decreased in a dose dependent manner with ~35% remaining at 1μM. Other than Na amplitude, the effects were maximal for all of the endpoints at 0.316μM including arrhythmias. This compound also appears to have a slower action than many hERG inhibitors, with arrhythmias and maximal responses occurring after 3 hours of treatment. These results suggest that the use of stem cell derived cardiomyocytes in physiological based assays can improve the prediction of cardiac liabilities.

Introduction

- Late stage failures of drugs in clinical trials have significant costs as well as significant safety risk to patients.
- Identification of liabilities early will save money and allow for prioritization of better compounds.
- Vanoxerine failed in a Phase 3 clinical trial where it caused 3 cases of ventricular arrhythmias out of 26 patients treated (3/26, 11.5%).(1)
  - Previous clinical trials had no torsades de pointes. (2)
- Can we identify this risk using iPSC derived human cardiomyocytes?
- What concentrations should we test?
  - At the dose used (400mg) Total Cmax is ~831 (3,4)
  - In vitro plasma protein binding is greater than 99%.
  - hERG is a high affinity target while plasma protein is probably not.
  - hERG is on the external surface of cells, therefore it is exposed to the plasma bound Vanoxerine. Compound should be accessible to the target since it is not covalently bound and will have an on/off rate.
- What timepoints should be tested?
  - Many hERG inhibitors act immediately on cells to cause arrhythmic effects while other compounds may have delayed effects.
- Due to differential signals from different ion channels, are the expected effects going to be purely dose dependent or will there be optimal concentrations where the effects are more prominent?
- What ADME characteristics of Vanoxerine may help to predict liabilities
  - Rapid microsomal clearance
  - Clearance is through CYP3A4 (5)
  - Compound is highly PP bound
  - Could there be a drug drug interaction (DDI) increasing the risk

Methods

- 48-well MEA plates were pre-coated with 5μl of fibronectin directly over the electrode grid and incubated at 37°C one hour before plating cells.
- iCell² cardiomyocytes were then resuspended in CDI Cardiomyocyte plating medium and dot plated in 5μl at a density of 50,000 viable cells per well.
- The cells were incubated, humidified at 37°C in 5% CO_2 for 2 hours.
- 300μL of CDI cardiomyocytes maintenance medium was slowly added to each well in 2 -150μl additions to avoid detaching the cells.
- 100% of the medium was changed to 500μl maintenance medium after 2 days.
- Cells are ready for testing after an additional 3 days. Medium was changed 24 hours prior to testing.
- Compounds are serially diluted in DMSO at 500X the concentrations to be tested. Compound is diluted 50 fold in an intermediate plate followed by addition of compound in medium from the intermediate plate at a 10 fold dilution into the MEA plate (500 fold dilution).
- Recordings were acquired before compound treatment (baseline) and after dosing (1 hour and 2 hours).

MEA Results

Treatment	Beat Period	Spike Slope	Spike Amplitude	Field Potential Duration (FPD)	Comments
1μM Vanoxerine	103 ± 0.7%	33 ± 5.0%	38 ± 5.0%	120 ± 4.4%	
316nM Vanoxerine	115 ± 2.9%	63 ± 7.8%	65 ± 9.3%	157 ± 7.3%	Arrhythmia
100nM Vanoxerine	110 ± 0.7%	87 ± 4.1%	88 ± 3.2%	145 ± 1.7%	
31.6nM Vanoxerine	106 ± 0.7%	95 ± 3.7%	95 ± 3.8%	114 ± 5.2%	
10nM Vanoxerine	102 ± 0.9%	98 ± 8.9%	97 ± 7.6%	107 ± 2.1%	

Table 1. MEA results for Vanoxerine. Data reported was for a 90 minute recording which began at 2 hours and ended at 3.5 hours post treatment. FPD is shown using the Matlab FPD tool provided by Axion. All data is the average of 5 to 7 wells.

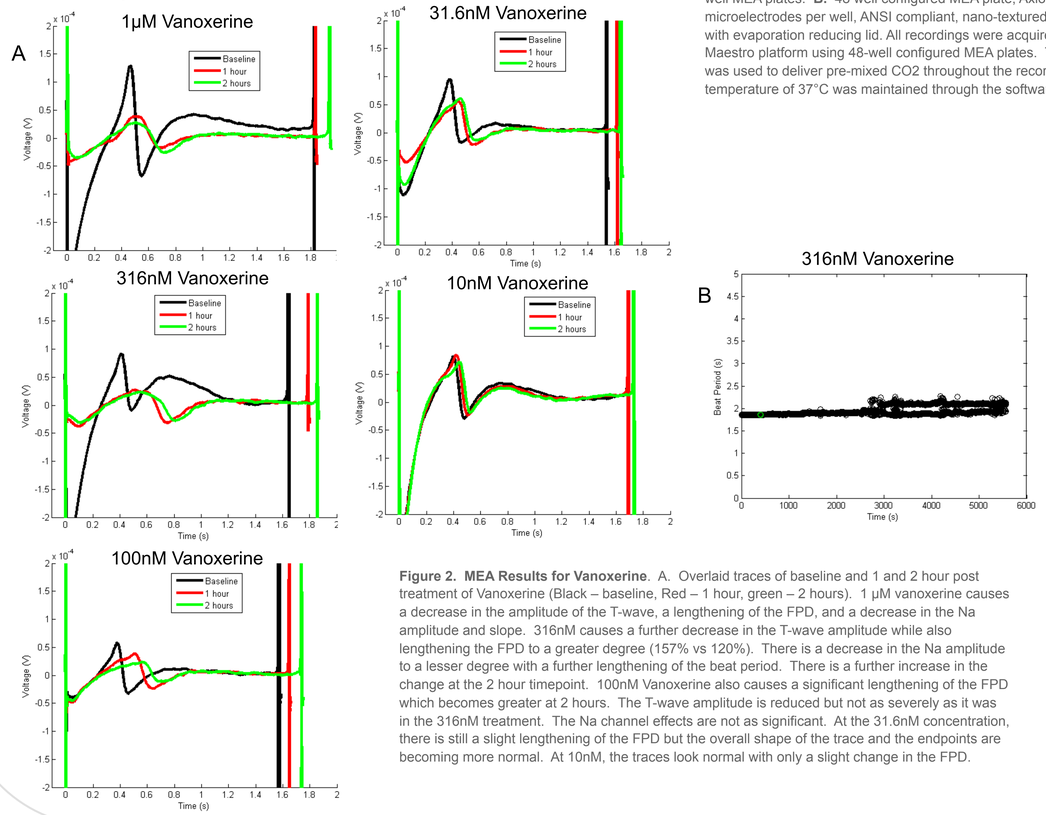


Figure 2. MEA Results for Vanoxerine. A. Overlaid traces of baseline and 1 and 2 hour post treatment of Vanoxerine (Black – baseline, Red – 1 hour, green – 2 hours). 1 μM vanoxerine causes a decrease in the amplitude of the T-wave, a lengthening of the FPD, and a decrease in the Na amplitude and slope. 316nM causes a further decrease in the T-wave amplitude while also lengthening the FPD to a greater degree (157% vs 120%). There is a decrease in the Na amplitude to a lesser degree with a further lengthening of the beat period. There is a further increase in the change at the 2 hour timepoint. 100nM Vanoxerine also causes a significant lengthening of the FPD which becomes greater at 2 hours. The T-wave amplitude is reduced but not as severely as it was in the 316nM treatment. The Na channel effects are not as significant. At the 31.6nM concentration, there is still a slight lengthening of the FPD but the overall shape of the trace and the endpoints are becoming more normal. At 10nM, the traces look normal with only a slight change in the FPD.

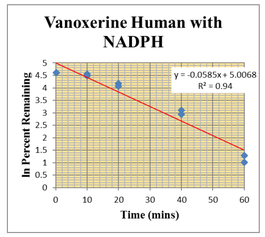
Axion BioSystems' Maestro Microelectrode Array (MEA) Platform

Figure 1. A. The Maestro, Axion Biosystems. 768 recording channels with fully integrated heater and software controls. Accommodates 12, 48 and 96 well MEA plates. **B.** 48 well configured MEA plate, Axion Biosystems. 16 microelectrodes per well, ANSI compliant, nano-textured gold electrodes with evaporation reducing lid. All recordings were acquired on the Axion Maestro platform using 48-well configured MEA plates. The Axion ECmini was used to deliver pre-mixed CO2 throughout the recordings. A Constant temperature of 37°C was maintained through the software controller.

ADME Results

Microsomal Clearance

A



Measurement	Value
Slope	-0.0585
Intercept	5.01
R^2	0.940
$T_{1/2}$ (min)	11.8
Microsome Conc. (mg/ml)	0.3
CL_{int}	195

Protein Binding in FBS

B

Test Article	Conc.	Mean FBS Fraction Unbound	Mean FBS Fraction Bound	Post Assay Recovery
Vanoxerine	100nM	0%	100%	45.1%
Vanoxerine	316nM	0%	100%	60.3%

Figure 3. ADME results. A. Microsomal clearance was performed on Vanoxerine which showed a rapid clearance with a Cl_{int} of 195 and a compound half life of 11.8 minutes. This would suggest that Vanoxerine would be rapidly cleared. **B.** Vanoxerine has been reported to be highly bound in human plasma. Human PPB was performed here and showed close to 100% binding. We repeated this assay in the CDI cardiomyocytes medium that contains 10% FBS at the relevant concentrations an showed that it is also close to100% bound in the medium.

Conclusions

- Use of the MEA and CDI iPSC derived cardiomyocytes identified a proarrhythmic liability as well as effects on the field potential duration.
- At 100 and 316nM, the compound caused a significant lengthening of the FPD of greater than 40%. There was a lengthening at the other doses also but they were significantly less. Even 1μM caused a less severe lengthening.
- There is a dose dependent decrease in NA amplitude and slope.
- There was a significant flattening of the T-wave at the top 3 concentrations suggesting hERG block.
- At the 316nM concentration, Vanoxerine caused arrhythmia after about 3 hours in 4 out of 7 wells. 3 hours is a delayed response not usually associated with hERG induced arrhythmia.
- The Cmax for Vanoxerine with a 400mg dose which was used in the clinical trial was ~800nM. This would be in the range of effect for the in vitro assay.
- Although plasma protein binding is high for Vanoxerine, in the presence of a high affinity target such as hERG, drug would be expected to find the target due to the on/off rate of the molecule. PPB is not covalent.
  - PPB was done in FBS containing CDI medium and was found to be close to 100% bound
- With the slow induction of arrhythmia on the cardiomyocytes, the high clearance rate may suggest that the compound would not be maintained at high enough levels for the liability. This may have made previous trials for Vanoxerine demonstrate safety. Further analysis of the patients in this trial may be warranted to determine possible DDI effects. Due to the 3A4 clearance of Vanoxerine, it is possible that the patients may have been on another drug that was a 3A4 inhibitor.

References

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