Parametric longitudinal analysis characterized the relationship between the concentration of a CK1δ inhibitor and the elongation of a cell circadian cycle

Ondrej Libiger and Jonathan Shelton

Janssen R&D, 3210 Merryfield Row, La Jolla, CA, 92121

INTRODUCTION

Recent clinical data highlighted the reciprocal link between circadian disruption and psychiatric disorders[1]. Casein kinase 1 delta (CK1δ) is an enzyme that helps regulate the internal pacemaker of cells[2]. Inhibiting its activity has the potential to restore circadian rhythms in patients with bipolar disorders, schizophrenia and depression[3,4,5].

OBJECTIVE

Characterize the relationship between compound concentration and the elongation of the circadian cycle using real-time monitoring of photon production in U2OS cells transfected with the circadian transcription factor BMAL linked to luciferase (“Clock in a Dish”).

METHODS

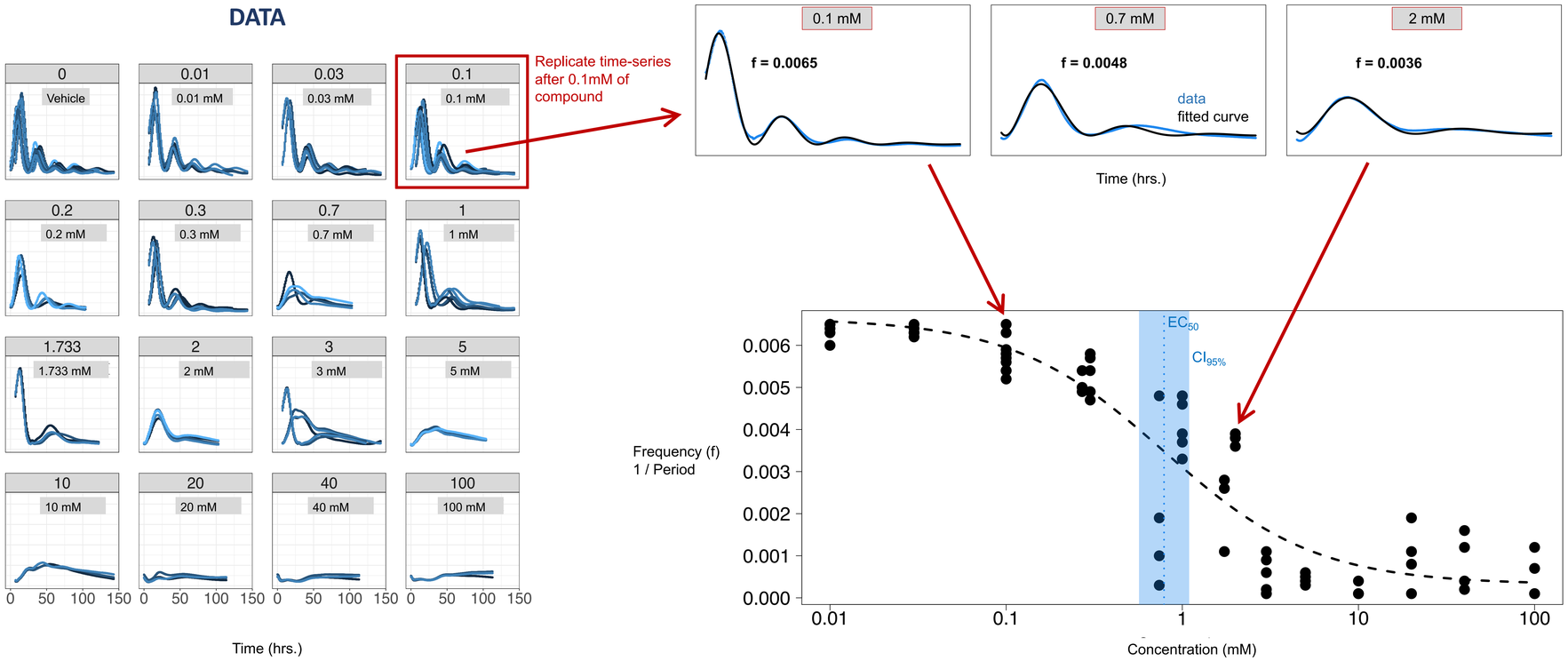
Multiple independent time series measurements spanning five days were taken at five-minute increments for various concentrations of a tool compound with documented potency, yielding ~60,000 data points. Consistent with literature, the observed data indicated the presence of circadian rhythms with longer cycle periods as the concentration of the compound increased. To quantify the magnitude of this effect, we fitted a nonlinear cyclical curve defined by a displacement offset (C), peak amplitude (A), phase angle (φ), cycle period (1/f) and a decay constant (τ) to each time series using the Levenberg-Marquardt algorithm. We then characterized the relationship between the compound’s concentration and cycle period by fitting a 3-parameter logistic function to the resulting cycle frequency estimates.

Peak amplitude *Frequency (1/Period)* *Displacement offset*

$$y(t) = \mathrm{A} \cdot e^{-\tau t} \cdot \sin(2\pi f t + \varphi) + e^{-\tau t} + C$$

Decay constant *Phase angle*

DATA



RESULTS

The tool compound’s half maximal effective concentration (EC_{50}) was estimated at 0.8 mM ($CI_{95\%}$: 0.6-1.1). A custom R script implementing the described two-step nonlinear parametric longitudinal analysis has subsequently been used to rapidly screen multiple compounds for relative potency.

CONCLUSIONS

Combining the automated analysis with the “Clock in a Dish” model facilitated the decision-making process regarding whole cell permeability and potency of novel CK1δ inhibitors, and thus supported an active discovery program within the Neuroscience therapeutic area.

REFERENCES

1) McClung, C.A. (2013). How Might Circadian Rhythms Control Mood? Let Me Count the Ways... Biol. Psychiatry *74*, 242–249.

2) Xu, Y., Padiath, Q.S., Shapiro, R.E., Jones, C.R., Wu, S.C., Saigoh, N., Saigoh, K., Ptáček, L.J., and Fu, Y.-H. (2005). Functional consequences of a CKIδ mutation causing familial advanced sleep phase syndrome. Nature *434*, 640–644.

3) Edgar, N., and McClung, C.A. (2013). Major depressive disorder: A loss of circadian synchrony?: Prospects & Overviews. BioEssays *35*, 940–944.

ACKNOWLEDGEMENTS

We thank Zhenya Cherkas, Mariusz Lubomirski and Javier Cabrera and for statistical advice.

Contact: OLibiger@its.jnj.com