Human iPSC-derived neurons for functional assessment of in vitro neurotoxicity and seizure liability

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BACKGROUND

In vitro pharmacology profiling of new chemical entities during early phases of drug discovery has recently become an essential tool to predict clinical adverse effects. While for cardiac safety testing high technology platforms are available, specific in vitro neurotoxic panels are not, and in vivo models are used instead. However, correlations between animal and human data are often weak; in addition, animal studies are expensive, ethically questionable and require large amounts of chemical compounds.

We have developed assays to assess in vitro neurotoxicity in a human system based on two different types of human induced pluripotent stem cell (iPSC)-derived cells and multielectrode array (MEA) technology.

Peri.4U are iPSC-derived peripheral neurons that reveal clear detectable burst-like activity after 3-4 day culture on MEA chips, indicating the presence of adverse effects. While for cardiac safety testing high technology platforms are available, specific in vitro neurotoxic panels are not, and in vivo models are used instead. However, correlations between animal and human data are often weak; in addition, animal studies are expensive, ethically questionable and require large amounts of chemical compounds.

RESULTS

PERI.4U FOR NEUROTOXICITY ASSESSMENT

PERI.4U exhibit long-term burst-like spontaneous activity. 16 electrodes of a 48-well MEA measurement (A). 100 % of wells revealed burst activity over c. 3 weeks (B).

Characteristics of CNS.4U (Fig. 1):

CNS.4U form extensive neuronal networks (A) and exhibit long-term synchronous network activity assessed by MEA (B). Network bursts representing synchronous spontaneous activity assessed by MEA technology over c. 5 weeks in culture. Here, we provide proof-of-concept results that reveal the suitability of CNS.4U™ for seizure liability assays based on dose-dependent reactivity to compounds that are known to affect seizure.

MEASUREMENT OF NEUROTOXICITY

The suitability of CNS.4U™ for neurotoxicity and seizure liability assessment was investigated in vitro using MEA technology.

METHODS

- Peri.4U and CNS.4U were plated in PEI coated 48-wells MEA chips from Axion Biosystems (Maestro System).
- 7 2 x 10⁴ cells were seeded per well in 3 µl droplets
- After 3-4 days in culture, both neuron types are spontaneously active
- MEA recordings were performed with cells that had been cultured for up to 3 weeks (Peri.4U) or up to 8 weeks (CNS.4U)
- Drugs were diluted in medium and applied as single dose or cumulatively in increasing concentrations. During drug application, 10% of the bath solution was replaced with a 10-fold concentrated drug solution.

CONCLUSIONS

- MEA assays for two different types of iPSC-derived neuronal cell types have been established
- For both Peri.4U and CNS.4U, burst-like activity can be measured on MEA with high reproducibility
- Peri.4U in combination with MEA technology comprise a suitable and predictive test system to assess neurotoxic compound effects
- Proof-of-concept experiments reveal suitability of CNS.4U™ for seizure liability assays given their long-term synchronous network activity and reactivity to seizure-active compounds

OPERATION PARTNER

Pharmacia

FOR NEUROTOXICITY ASSESSMENT

PERI.4U in combination with MEA technology comprise a suitable and predictive test system to assess neurotoxic compound effects. Peri.4U data is in good agreement with published data (indicated in red).

Characteristics of CNS.4U™ (Fig. 1): CNS.4U form extensive neuronal networks and exhibit long-term synchronous network activity assessed by MEA technology.

CNS.4U exhibit burst-like spontaneous activity, synchronized over 16 electrodes of a 48well MEA measurement (C). Network bursts representing synchronous spontaneous activity assessed by MEA technology over c. 5 weeks in culture. Here, we provide proof-of-concept results that reveal the suitability of CNS.4U™ for seizure liability assays based on dose-dependent reactivity to compounds that are known to affect seizure.

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