Introduction

The functional network of human induced pluripotent stem cell (hiPSC)-derived neurons is a potentially powerful in vitro model for evaluating drug toxicity. Epilepticiform activity is one of phenomena in neuronal toxicity. To evaluate the dynamics of epileptiform activities and the effect of anti-convulsant drug in cultured hiPSC-derived neurons, we used the high-throughput miniature electrode array (MEA) system, where we simultaneously recorded extracellular potentials for 16 channels per well across 24-well plates. We examined chemically evoked epileptiform activities. Epilepticiform activities were induced by 4-Aminopyridine (4-AP), Pilocarpine, chlorpromazine, and pentyleneetetrazole (PTE). The number of synchronized burst firings were increased in a concentration dependent manner at 4-AP, Pilocarpine, and Chlorpromazine administration. On the other hand, the duration and spikes in a synchronized burst were increased at PTZ administration. Phenytion used in anti-convulsant drug suppressed electrophysiological activities. From these results, we suggest that the electrophysiological assay in cultured human iPSC-derived neuron using MEA system has the potential to investigate the neuronal toxicity in drug screening.

Material & Methods

Human iPSC-derived neurons and astrocytes

Human iPSC-derived cortical neurons (Axel Bioscience Science) were cultured 8000 cells/cm² on the MEA. hiPSC were cultured with astrocytes as feeder layer (Axel Bioscience). We prepared the cultures with astrocytes sample and only neurons culture samples.

To investigate pharmacological effects, we administered 4-aminopyridine (0, 0.3, 1, 3, 10, 30 µM), Pilocarpine (0, 1, 3, 10, 30 µM), Phenytoin (0, 1, 3, 10, 30, 100 µM) and Phenylhydantoin (0, 1, 3, 10, 30, 100 µM). In this way, we observed the changes in spontaneous firing at each concentration.

Result 1 Induction of epileptiform activities and an effect of anti-convulsant drug in co-cultured hiPSC-derived cortical neurons with astrocytes

Fig. 1. Laster plots and array-wide spike detection rate (AWSDR, spikes/100ms) at same well. (A) 4-aminopyridine (0, 0.3, 1, 3, 10, 30 µM). (B) Pilocarpine (0, 1, 3, 10, 30 µM). (C) Chlorpromazine (0, 1, 3, 10, 30, 100 µM). (D) Acetaminophen (0, 1, 3, 10, 30, 100 µM). (E) Phenylhydantoin (0, 1, 3, 10, 30, 100 µM). (F) Isoniazid (0, 1, 3, 10, 30, 100 µM).

The number of synchronized burst firings were increased in a concentration dependent manner at 4-AP, Pilocarpine, and Chlorpromazine administration. Accordingly, the duration and spikes in a synchronized burst were decreased. Significant changes were not observed at Isoniazid and Acetaminophen administration. Synchronized burst firings disappeared at 100 µM Phenytion administration.

Result 2 Induction of epileptiform activities in cultured hiPSC-derived cortical neurons

Fig. 2. The changes of firing properties in drug administration. (A) Total spikes rate v.s. 0 µM. (B) No. of synchronized burst firings v.s. 0 µM. (C) Duration in a synchronized burst v.s 0 µM. (D) No. of spikes in a synchronized burst v.s 0 µM.

Although the firing rate is different, response properties in only neurons culture sample were almost the same as co-culture sample at 4-AP, pilocarpine and chlorpromazine administration.

Conclusion

In conclusion, we detected epileptiform activities using typical convulsants in cultured hiPSC-derived neuronal networks and found the differences of response properties depending on the type of convulsants. Although the firing rate is different, response properties in only neurons culture sample were almost the same as co-culture sample at 4-AP, pilocarpine and chlorpromazine administration. High-throughput MEA system in cultured hiPSC-derived neurons proved useful for toxicological assays with electrophysiological functions.

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SPS 2017, Berlin Germany, 24 Sep —27 Sep