

# Oral Presentation 1

日時: 2020年10月29日 15:45-17:15  
チャンネル: 1

## 口頭発表1

『臨床応用(その他)/レギュラトリエンス』  
Clinical application/Regulatory Science

座長: 石田 誠一 (崇城大学)  
Seiichi Ishida (Sojo University)

### O1-01

“Evaluation of the expression profile of diarrhea induced by irinotecan using the Japanese Adverse Drug Event Report Database”

Mashiro Okunaka

(Meiji Pharmaceutical University / National Cancer Center Hospital East)

### O1-02

“Development of in vitro Blood Brain Barrier model reproducing microglia-induced cytokine/chemokine dynamics”

Kaoru Sato

(Lab Neuropharmacol, Div Pharmacol, NIHS)

### O1-03

“Establishment of culture protocol for application of human iPS cell-derived hepatocytes to cholestasis toxicity test”

Shinichiro Horiuchi

(National Institute of Health Sciences)

### O1-04

“Long term culture using hepatocytes from chimeric mice with humanized livers for toxicity tests”

Yukie Kuroda

(National Institute of Health Sciences)

# Evaluation of the expression profile of diarrhea induced by irinotecan using the Japanese Adverse Drug Event Report Database

**Mashiho Okunaka**<sup>1, 2</sup>  
mokonaka@east.ncc.go.jp.

**Daisuke Kano**<sup>2</sup>  
dkano@east.ncc.go.jp.

**Reiko Matsui**<sup>2</sup>  
rmatsui@east.ncc.go.jp.

**Yoshihiro Uesawa**<sup>1</sup>  
uesawa@my-pharm.ac.jp

<sup>1</sup> Department of Medical Molecular Informatics, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan

<sup>2</sup> Department of Hepatobiliary & Pancreatic Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa Chiba 277-8577, Japan

**Keywords:** Irinotecan; Diarrhea; Chemotherapy; JADER

**Background** Irinotecan (CPT-11) has been used in chemotherapy for advanced and recurrent colorectal cancer and diarrhea is one of the dose-limited toxicities of CPT-11 [1-4]. Currently, the differences in time of onset of diarrhea expression among CPT-11-containing regimens have not been fully clarified. Therefore, we investigated the tendency for CPT-11-induced diarrhea using the Japanese Adverse Drug Event Report (JADER) database published by the Pharmaceuticals and Medical Devices Agency [5].

**Method** The drugs selected for this investigation were CPT-11, CPT-11 plus fluorouracil (5-FU), and CPT-11 plus S-1, which are among the drugs listed in the JADER. We calculated the period starting from the beginning of onset of the adverse events. Moreover, we performed a time-to-onset analysis using the Weibull distribution, and examined the expression profile of adverse events for each drug.

**Results** A total of 15,563 reports on adverse events with CPT-11 as the suspected drug were found. Furthermore, 1,069 cases of diarrhea were reported for all drugs. In the time-to-onset analysis, CPT-11 was the earliest expressed diarrhea, with a median onset of 5 days. On the other hand, diarrhea developed more slowly with CPT-11 plus S-1 than with the other treatment, with a median onset of 14 days ( $p < 0.05$ ).

**Discussion** These results, which show that the time of onset of diarrhea varies greatly depending on the CPT-11-containing regimen, are expected to contribute to the management of toxicity in cancer chemotherapy.

[1] National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology colon cancer. Version 4. 2020.

[2] Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2019 for the treatment of colorectal cancer.

[3] Van Cutsem E, Cervantes A, Nordlinger B, Arnold D, et al. Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2014;25(3):1-9.

[4] [http://www.mochida.co.jp/dis/interview/iri\\_n7.pdf](http://www.mochida.co.jp/dis/interview/iri_n7.pdf)

[5] <https://www.pmda.go.jp/safety/info-services/drugs/adr-info/suspected-adr/0003.html>

# Development of in vitro Blood Brain Barrier model reproducing microglia-induced cytokine/chemokine dynamics

**Kaoru Sato**<sup>1</sup>  
kasato@nihs.go.jp

**Kazue Hoshikawa**<sup>1</sup>  
hoshikawa@nihs.go.jp

**Yukari Shigemoto-Mogami**<sup>1,2</sup>  
shigemoto@nihs.go.jp

<sup>1</sup> Laboratory of Neuropharmacology, Division of Pharmacology, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki city Kanagawa 210-9501, Japan

<sup>2</sup> Department of Neuropharmacology, Interdisciplinary Graduate School of Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan

**Keywords:** BBB, Microglia, Cytokine, Chemokine

The blood-brain barrier (BBB) restricts the transfer of substances between blood and brain tissue thereby protecting the brain from harmful substances. In the drug development, the BBB barrier function is important in terms both of the drug delivery and the diagnosis of the pathological changes. We therefore attempted to establish the in vitro BBB model reflecting the pathological permeability changes. We here focused on microglia, the cells playing key roles in neuroinflammation.

In this study, we employed in vitro BBB model composed of vascular endothelial cells (EC), pericytes (Peri), and astrocytes (Ast), and then investigated the contribution of microglia to BBB barrier function via cytokine/chemokines (C/C). When non-stimulated microglia (non-MG) or LPS-activated microglia (LPS-MG) was added to the abluminal side of the in vitro BBB model, the BBB barrier function was disrupted only when activated microglia was added. LPS-MG caused decreases in the trans-endothelial electrical resistance (TEER) and in the expression levels of tight junction (TJ) proteins. Under these conditions, 19 C/Cs were markedly increased on the abluminal side. Unexpectedly, although LPS-MG alone released 10 of the 19 C/Cs, their concentrations were much lower than those detected on the abluminal side of the BBB model with LPS-MG. Co-culture of LPS-MG with Ast caused marked increases in 12 of the 19 C/Cs, while co-culture of LPS-MG with EC and Peri resulted in a significant increase in only 1 of the 19 C/Cs (fractalkine). These results suggest that C/C dynamics in this experiment model are not only caused by activated microglia but also are due to the interaction between activated microglia and astrocytes. Taken together, microglia is indispensable to in vitro BBB model reproducing the complex interactions among neurovascular unit (NVU) cells and pathological changes via C/Cs in the progressive process of neuroinflammation.

[1] Shigemoto-mogami Y., *et al.*: *Frontiers in Cellular Neuroscience*, 12: 494, 2018

[2] Shigemoto-mogami Y. and Sato K.: *Folia Pharmacologica Japonica Nippon Yakurigaku Zasshi*, (in press).

## Establishment of culture protocol for application of human iPS cell-derived hepatocytes to cholestasis toxicity test

**Shinichiro Horiuchi<sup>1</sup>**    **Yukie Kuroda<sup>1</sup>**    **Su-Ryang Kim<sup>1</sup>**  
hori542@nihs.go.jp

**Ryuya Fujii<sup>1</sup>**    **Seiichi Ishida<sup>1,2</sup>**

<sup>1</sup> Division of Pharmacology, National Institute of Health Sciences,  
3-25-26 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa, 210-9501, Japan

<sup>2</sup> Sojo University, Faculty of Biotechnology and Life Science  
4-22-1 Ikeda, Nishi-ku, Kumamoto, Kumamoto, 860-0082, Japan

**Keywords:** human iPS cell-derived hepatocytes, drug induced cholestasis toxicity, sandwich culture, bile canaliculi, drug metabolism

[Purpose] Drug-induced liver injury (DILI) is one of the primary reasons for the failure of pharmaceutical agents during drug development. 40% of DILI cases in Japan had either cholestatic or mixed cholestatic hepatic toxicity (1) and fatality rate of the case reached 10 - 50% (2). Accordingly, evaluation for the drugs that cause cholestasis is important in drug development. Primary/cryo-preserved human hepatocytes is used to test the toxicity of candidate compounds in the early stages of drug development. However, primary/cryo-preserved human hepatocytes is not considered suitable for the evaluation of cholestasis toxicity because the extended bile canaliculi formation is difficult. In our previous experiments, the extended bile canaliculi were observed on a part of cell culture surface when hiPSC cell-derived hepatocytes (hiPSC-hep) were cultured for long term. Therefore, we studied better culture conditions for functional extended bile canaliculi formation, aiming the application of hiPSC-hep to evaluation for cholestasis toxicity.

[Method] hiPSC-hep from vendor A was used in this study. hiPSC-hep were sandwich-cultured after long term culture for formation of extended bile canaliculi. Bile canaliculi were observed using fluorogenic substrates of biliary efflux transporters MRP2 or BSEP. In addition, localization of MRP2 and BSEP, effect of biliary efflux transporter inhibitor and expression of major cytochrome P450 (CYPs) were observed.

[Results and Discussion] Extended bile canaliculi were evenly formed on the whole cell culture surface by examining culture condition. The localizations of MRP2 and BSEP in the canalicular membrane were confirmed by immunostaining and the excretion of model substrates to bile canaliculi were inhibited by corresponding inhibitors. These results suggested that functional bile canaliculi were formed. Expressions of major CYPs were same level as or slightly lower than cryopreserved human hepatocyte. From above results, culture protocol that was established in this study is expected to apply to drug induced cholestasis toxicity test.

[1] Takikawa, H., Murata, Y., Horiike, N., Fukui, H., Onji, M., Drug-induced liver injury in Japan: An analysis of 1676 cases between 1997 and 2006, *Hepatol Res.*, 39(5):427-431, 2009.

[2] Navarro, VJ., Senior, JR., Drug-related hepatotoxicity, *N Engl J Med.*, 354(7):731-739, 2006

# Long term culture using hepatocytes from chimeric mice with humanized livers for toxicity tests

**Yukie Kuroda**<sup>1</sup> **Su-Ryang Kim**<sup>1</sup> **Shinichiro Horiuchi**<sup>1</sup> **Ryuya Fujii**<sup>1</sup>  
y-kuroda@nihs.go.jp

**Hiroshi Suemizu**<sup>2</sup> **Seiichi Ishida**<sup>1,3</sup>

<sup>1</sup> Division of Pharmacology, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki city Kanagawa 210-9501, Japan

<sup>2</sup> Department of Laboratory Animal Research, Central Institute for Experimental Animals, 3-25-12 Tonomachi, Kawasaki-ku, Kawasaki city Kanagawa 210-0821, Japan

<sup>3</sup> SOJO University, Faculty of Biotechnology and Life sciences, 4-22-1 Ikeda, Nishi-ku Kumamoto city Kumamoto 860-0082, Japan

**Keywords:** Chimeric mice, Hepatocyte, Gene expression, Cytochrome P450, Transporter

[Purpose] In the assessment on acute and chronic influence of drug or chemical exposure to human body, *in vitro* test has been required continuous improvement for pharmacokinetic prediction in the body. Chimeric mice with humanized hepatocytes are constructed by transplanting human hepatocytes to severely immunodeficient hepatopathy mice. Hepatocytes isolated from chimeric mice expressed human albumin, human cytochrome P450 and human transporter, and expression levels of P450 subfamily mRNA correlated to those of human hepatocytes [1]. Thus, hepatocytes from chimeric mice with humanized liver are thought to be effective cell resource for metabolic activity evaluation and for toxicity assays.

In this study, we tried to culture hepatocytes from chimeric mice with humanized livers for the longer period to apply them to repetitive toxicity tests in the future.

[Materials and Methods] Hepatocytes from chimeric mice with humanized livers (Hu-Liver cells) prepared at Central Institute for Experimental Animals were cultured with hiPS-HEP LTM Medium (LTMM) (Takara Bio Inc., Shiga, Japan), Hepatocyte maintenance medium (HMM) (Thermo Fisher Scientific, Waltham, MA), or mixed medium of these two on the collagen coated plate or on 3-dimensional cell culture plate Cell-able<sup>®</sup> (Toyo Gosei Co., Ltd, Tokyo, Japan).

[Results and Discussion] Gene expression level of transporters in Cell-able<sup>®</sup> or HMM culture conditions were equal to those in the cells right after the preparation from chimeric mice. CYP3A4 activity and drug metabolizing enzyme gene expression level showed higher in LTMM condition than in others. Cell polarity was observed in HMM and LTMM with collagen plate conditions. So, these cell culture conditions using Hu-Liver were able to improve or maintain cell functions. In this presentation, we are planning to report the results of cell culture condition improvement for the toxicity tests.

- [1] S. Uehara, *et al.* Expression and inducibility of cytochrome P450s in human hepatocytes isolated from chimeric mice with humanised livers. *Xenobiotica*, 49.6: 678-687, 2019.