Antiviral Research 123 (2015) 120–131

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Modeling rotavirus infection and antiviral therapy using primary intestinal organoids

Yuebang Yin [a], Marcel Bijvelds [a], Wen Dang [a], Lei Xu [a], Annemiek A. van der Eijk [b], Karen Knipping [c], Nesrin Tuysuz [d], Johanna F. Dekkers [e], Yijin Wang [a], Jeroen de Jonge [f], Dave Sprengers [a], Luc J.W. van der Laan [f], Jeffrey M. Beekman [e], Derk ten Berge [d], Herold J. Metselaar [a], Hugo de Jonge [a], Marion P.G. Koopmans [b], Maikel P. Peppelenbosch [a], Qiuwei Pan [a,*]

[a] Department of Gastroenterology and Hepatology, Erasmus MC-University Medical Center, Rotterdam, The Netherlands
[b] Department of Viroscience, Erasmus MC-University Medical Center, Rotterdam, The Netherlands
[c] Nutricia Research, Utrecht, The Netherlands
[d] Department of Cell Biology, Erasmus MC Stem Cell Institute, Erasmus MC-University Medical Center, Rotterdam, The Netherlands
[e] Department of Pediatric Pulmonology/Laboratory of Translational Immunology, Wilhelmina Children's Hospital, University Medical Centre, Utrecht, The Netherlands
[f] Department of Surgery, Erasmus MC-University Medical Center, Rotterdam, The Netherlands

ARTICLE INFO

Article history:
Received 19 June 2015
Revised 18 September 2015
Accepted 19 September 2015
Available online 25 September 2015

Keywords:
Rotavirus
Intestinal organoids
Interferon
Ribavirin

ABSTRACT

Despite the introduction of oral vaccines, rotavirus still kills over 450,000 children under five years of age annually. The absence of specific treatment prompts research aiming at further understanding of pathogenesis and the development of effective antiviral therapy, which in turn requires advanced experimental models. Given the intrinsic limitations of the classical rotavirus models using immortalized cell lines infected with laboratory-adapted strains in two dimensional cultures, our study aimed to model infection and antiviral therapy of both experimental and patient-derived rotavirus strains using three dimensional cultures of primary intestinal organoids. Intestinal epithelial organoids were successfully cultured from mouse or human gut tissues. These organoids recapitulate essential features of the *in vivo* tissue architecture, and are susceptible to rotavirus. Human organoids are more permissive to rotavirus infection, displaying an over 10,000-fold increase in genomic RNA following 24 h of viral replication. Furthermore, infected organoids are capable of producing infectious rotavirus particles. Treatment of interferon-alpha or ribavirin inhibited viral replication in organoids of both species. Importantly, human organoids efficiently support the infection of patient-derived rotavirus strains and can be potentially harnessed for personalized evaluation of the efficacy of antiviral medications. Therefore, organoids provide a robust model system for studying rotavirus–host interactions and assessing antiviral medications.

1. Introduction

Rotavirus, a member of the *Reoviridae* family, is a leading cause of severe gastroenteritis in young children worldwide. It yearly causes 1.4 billion diarrhea episodes and over 450,000 deaths of children under five years of age (Anon., 2013). Although infections in adults are often asymptomatic or mild, emerging evidence indicates that organ transplantation patients, both pediatric and adult organ recipients, are particularly sensitive to rotavirus infection with severe gastroenteritis as well as other complications (Lee and Ison, 2014). Unfortunately, no specific antiviral drug against rotavirus is available except supportive care (Poppitt et al., 2014). The potential off-label use of general antiviral drugs, such as interferon-alpha (IFN-α) and ribavirin, have been poorly investigated (Wang et al., 2014).

The absence of a robust experimental *in vitro* system allowing investigation of rotavirus infection is a bottleneck hampering development of novel rational strategies aiming at combating rotavirus-associated diseases. It is well-known that rotavirus

Abbreviations: IFN-α, interferon-alpha; dsRNA, double stranded RNA; CF, cystic fibrosis; IHC, immunohistochemistry; qRT-PCR, quantitative real-time polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; VP, viral protein; ISG, interferon-stimulated gene; IRF, interferon regulatory factor; IFITM, interferon-induced transmembrane protein; STAT, signal transducer and activator of transcription; PKR, protein kinase R; HCV, hepatitis C virus; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; P/S, Penicillin/Streptomycin; CMGF-, complete medium growth factor-; CCS, complete chelating solution.

* Corresponding author at: Department of Gastroenterology and Hepatology, Erasmus MC, Room Na-617, 'sGravendijkwal 230, NL-3015 CE Rotterdam, The Netherlands.

E-mail address: q.pan@erasmusmc.nl (Q. Pan).

http://dx.doi.org/10.1016/j.antiviral.2015.09.010
0166-3542/

primarily infects complexly organized epithelium of the small intestine (Tam and Roner, 2011), while currently used cell culture models based on simple two dimensional (2D) cultures of immortalized cell lines (Chaibi et al., 2005; Finkbeiner et al., 2012; Knipping et al., 2012) that do not capture the dynamics nor individual variation existing in the patient mucosa (Chaibi et al., 2005; Knipping et al., 2012). Complicating further is the genomic diversity in patient-derived rotavirus strains. Because rotavirus is a double stranded RNA (dsRNA) virus containing eleven highly variable segments (Desselberger, 2014). In total, more than 110,000 strains have been genotyped originating from 100 different countries and relative prevalence of different strains is continuously evolving (van Maarseveen et al., 2010). In contrast, the difficulty in setting up *in vitro* models has led to the situation that only a limited number of well-adapted laboratory strains are almost exclusively used for experimental research. Therefore, current approaches for modeling rotavirus infection are far from satisfactory (Knipping et al., 2012), which prompts development of novel experimental avenues.

Three dimensional (3D) cultured primary intestinal organoids are currently innovating research of intestinal physiology and pathology. They contain various types of cells and recapitulate most if not all aspects of *in vivo* tissue architecture (Hynds and Giangreco, 2013; Lancaster and Knoblich, 2014; Sato and Clevers, 2013a). Accordingly, organoids have been successfully used to model a plethora of diseases including cystic fibrosis (CF, an intestinal disease that has been usually difficult with respect to *in vitro* investigation) (Dekkers et al., 2013) and cancer (Sachs and Clevers, 2014). In the present study, we explored the feasibility of using primary intestinal organoids to model rotavirus infection and antiviral therapy. We demonstrated that organoids, in particular of human origin, effectively support rotavirus infection of both laboratory-adapted and patient-derived strains, as well as production of infectious virus particles. Most interestingly, human organoids were able to assess the responsiveness of patient-derived rotavirus to different antivirals on an individual basis.

2. Materials and methods

2.1. Viruses

Simian rotavirus SA11, a well-characterized and broadly used laboratory strain was used (Cecilio et al., 2012). SA11 rotavirus used in the study was prepared as described previously (Knipping et al., 2012). Stool samples (stored at −80 °C freezer) from nine rotavirus patients were obtained from the Erasmus MC biobank, Department of Viroscience, Erasmus Medical Center, Rotterdam. These samples were taken during diarrhea period and tested for enterovirus, parechovirus, norovirus genogroups I and II, rotavirus, adenovirus, astrovirus and sapovirus by qRT-PCR described by previous studies (Corless et al., 2002; Hoek et al., 2013; van Maarseveen et al., 2010).

2.2. Cell culture

Caco2 cell line (Human Caucasian colon adenocarcinoma; ECACC) was cultured in Dulbecco's modified Eagle's medium (DMEM; Lonza, Verviers, Belgium) supplemented with 20% (vol/vol) heat-inactivated fetal calf serum (FCS; Sigma–Aldrich, St. Louis USA) and 100 U/ml of Penicillin–Streptomycin (Gibco, Grand Island, USA) solution. Cells were maintained in 5% CO_2 at 37 °C in a humidified incubator. Cell genotyping analysis was performed at the Department of Pathology, Erasmus Medical Center, Rotterdam. Mycoplasma was routinely examined in our laboratory by the MycoAlert™ Mycoplasma Detection Kit (Lonza, Rockland, ME USA) according to the manufacturer's instruction. All cells were confirmed to be mycoplasma negative.

2.3. Culture of primary mouse intestinal organoids

Mice were sacrificed by cervical dislocation following euthanasia with sodium pentobarbital (100 mg per kilogram body weight; IP injection). Subsequently, mouse small intestine (jejunum and ileum) was harvested. Stool was flushed out with ice-cold PBS. The small intestine was dissected and cut longitudinally and washed with ice-cold PBS. Villus was scraped off with a coverslip and the remaining parts of fat were removed. Small intestine was then cut into small pieces (0.2–0.5 cm) and transferred to a 50 ml tube, and washed 3 times with ice-cold PBS. Then, the tissues were rocked with 2 mM EDTA for 30 min at 4 °C. Afterwards, the tissues were thoroughly suspended by pipetting up and down for 10 times with a 10 ml tip in order to loosen crypts, and then were filtered through a 70 μm cell strainer. Crypt suspension was added with 10% (vol/vol) FCS and spin down at 300*g* for 5 min. Supernatant was discarded and crypts were re-suspended in 10 ml complete medium growth factor (GF)- (CMGF-, advanced DMEM/F12 was supplemented with 1% (vol/vol) of GlutaMAX™ Supplement (Gibco, Grand island, USA), 10 mM of Hepes and 100 U/ml of Penicillin–Streptomycin), and then crypts were collected by centrifugation at 150*g* for 2 min at 4 °C. Approximately 500 crypts were suspended in 40 μl growth factor reduced phenol-red free Matrigel (Corning, Bedford, USA). Afterwards, a 40 μl droplet of Matrigel/crypts mix was placed in the center of each well of a 24-well plate, and was subsequently incubated at 37 °C with 5% CO_2 for 15 min. 500 μl of culture medium was added per well after Matrigel got solidification. The culture medium was supplemented with CMGF-, 2% (vol/vol) of B-27® Supplements (Gibco, Grand Island, USA), 1% (vol/vol) of N2® Supplements (Gibco, Grand Island, USA), 500 pg/L of epidermal growth factor (EGF), 20% (vol/vol) of R-Spondin 1 (conditioned medium), and 10% (vol/vol) of Noggin (conditioned medium). The medium was maintained until passaging organoids (it depends on the density of organoids whether medium should be refreshed during culture). To this aim, organoids were removed from Matrigel and broken up mechanically by passing through a 5 ml tip inserting a 200 μl tip, and then were transferred to fresh Matrigel. The passaging was performed every 5–6 days with a 1:3 split ratio. Each well contains 10 or more organoids.

2.4. Culture of primary human intestinal organoids

Intestinal biopsies or surgically resected intestinal tissues were transferred into a 15 ml tube containing 10 ml complete chelating solution (CCS, MilliQ H_2O was supplemented with 1.0 g/L of Na_2HPO_4-$2H_2O$, 1.08 g/L of KH_2PO_4, 5.6 g/L of NaCl, 0.12 g/L of KCl, 15 g/L of Sucrose, 10 g/L of D-Sorbitol and 80 μg/L of DL-dithiothreitol). Biopsies/tissues were washed three times by pipetting up and down 8–10 times and then were rocked with 8 mM EDTA for 15 min at 4 °C. Supernatant with EDTA was discarded and 5 ml fresh CCS solution was added. It was thoroughly suspended by pipetting up and down with 10 ml tip for 8–10 times to loosen crypts, and 2 ml FCS was added. Supernatant with crypts was transferred into a 50 ml tube and biopsies were re-used for repeatedly collecting more crypts (2–3 times). Then, crypt suspension was centrifuged at 300*g* for 5 min. Supernatant was discarded and crypts were re-suspended in 2 ml CMGF-, and then crypts were collected by centrifugation at 130*g* for 5 min at 4 °C. Crypts were finally suspended in 40 μl growth factor reduced phenol-red free Matrigel (Corning, Bedford, USA). Then, a 40 μl droplet of Matrigel/crypt mix was placed in the center of each well of a 24-well plate, and was subsequently incubated at 37 °C with