Antiviral Research 132 (2016) 76–84

Contents lists available at ScienceDirect

Antiviral Research

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

Post-exposure antiviral treatment of norovirus infections effectively protects against diarrhea and reduces virus shedding in the stool in a mortality mouse model

Joana Rocha-Pereira [a], Abimbola O. Kolawole [b], Eric Verbeken [c], Christiane E. Wobus [b], Johan Neyts [a, *]

[a] KU Leuven, University of Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium
[b] Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, USA
[c] KU Leuven – University of Leuven, Department of Imaging & Pathology, Translational Cell & Tissue Research, Leuven, Belgium

ARTICLE INFO

Article history:
Received 8 April 2016
Received in revised form
27 May 2016
Accepted 27 May 2016
Available online 29 May 2016

Keywords:
Antiviral drugs
Mouse model
Diarrhea
Outbreak
Control and prevention

ABSTRACT

Noroviruses are a leading cause of gastroenteritis across the world in all age groups and are linked to increased hospitalization and mortality in children, the elderly and immunocompromised. The development of specific antiviral treatment for norovirus gastroenteritis is urgently needed. We explored in a mouse model whether an inhibitor of norovirus replication could be used therapeutically post murine norovirus (MNV)-infection of mice.

Using the MNV, we previously discovered that the viral polymerase inhibitor 2′-*C*-methylcytidine (2CMC) is able to protect against diarrhea and mortality in mice when used prophylactically and to block the transmission of MNV between mice. Here, we investigated whether 2CMC could be used therapeutically, starting treatment between 12 h and 3 days post-infection with 2CMC.

Post-exposure treatment of MNV-infected mice with 2CMC was efficient up to 2 days after infection, preventing norovirus-induced diarrhea, delaying and reducing MNV shedding in stool of treated mice. Rehydration of 2CMC-treated animals did not result in a further improvement of the disease evolution compared to antiviral treatment only. The presence of MNV antigens and inflammation in the small intestine of infected mice inversely correlated with the effectiveness of delayed antiviral treatment. Anti-MNV IgGs were detected in re-challenged mice 10 weeks after the first contact, these protected the mice from re-infection. We here demonstrate the benefit of antiviral treatment in ongoing norovirus infections.

1. Introduction

Human noroviruses are a leading cause of gastroenteritis across the world in all age groups. Infections with these viruses can be particularly severe for children < 5 years of age. They are second only to rotavirus as etiologic agents of childhood diarrhea in both low-/middle- and high-income countries (Chhabra et al., 2014; Ramani and Kang, 2009; Walker et al., 2013; Yu et al., 2015). In those countries where routine vaccination against rotavirus has been implemented, human norovirus has become the more commonly detected agent of childhood diarrhea (Hemming et al., 2013; Payne et al., 2013). Whereas single episodes of diarrhea are typically self-limiting and of short duration, several episodes per year can lead to nutritional deficits and long-term consequences, such as growth stunting. This important sequela, which is associated with decreased cognitive function, could (in ~25% of cases) be attributed to five or more episodes of diarrhea before the age of 2 (Walker et al., 2013). Whereas there are two vaccines on the market to prevent rotavirus-induced diarrhea, there is no vaccine or specific antivirals to prevent or treat norovirus-induced gastroenteritis. Supportive care consists mostly of electrolyte replenishment of dehydrated individuals. Today highly effective and potent antivirals are available for the treatment of infections with herpesviruses, HIV, hepatitis B and C and influenza viruses. There is no doubt that

* Corresponding author. KU Leuven – University of Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium.
E-mail address: johan.neyts@kuleuven.be (J. Neyts).

http://dx.doi.org/10.1016/j.antiviral.2016.05.022
0166-3542/

it should be possible to develop highly potent and safe inhibitors of noroviruses, provided sufficient efforts. Such drugs should allow the treatment of severe and prolonged diarrhea not only in children, but also among the elderly population for whom norovirus infections account for the majority of gastroenteritis-associated hospitalization and deaths (Hall et al., 2012; Lopman et al., 2011; Trivedi et al., 2012; van Asten et al., 2011) and for immunocompromised patients.

Murine norovirus (MNV) is a genogroup V norovirus that has been widely used as a surrogate for human noroviruses (Karst et al., 2003; Wobus et al., 2004). The recent report that a human B cell line can support the replication of the human virus and the development of a mouse model for human norovirus infection may bring new possibilities for future studies (Jones et al., 2014; Taube et al., 2013). We recently reported that a small-molecule inhibitor of norovirus replication – 2′-*C*-methylcytidine (2CMC) – is able to protect against diarrhea and mortality in alpha/beta (IFN-α/β) and gamma interferon (IFN-γ) receptor knockout AG129 mice when given prophylactically (Rocha-Pereira et al., 2013). Furthermore, we used MNV to develop a transmission model and provided evidence that MNV is efficiently transmitted from infected animals to sentinel mice (Rocha-Pereira et al., 2015). In this model, we demonstrated for the first time that transmission of norovirus is efficiently blocked by prophylactic treatment of the sentinel mice with 2CMC. Here, we explore whether therapeutic use of an inhibitor of norovirus replication (i.e., 2CMC) also results in a beneficial effect on norovirus infection in the infected host.

2. Materials and methods

2.1. Cells, viruses and compound

2′-*C*-methylcytidine (2CMC) was synthesized as described (Pierra et al., 2006) and dissolved in sterile saline. Lactated Ringer's Solution (LRS, Lactated Ringer's Injection USP, BBraun, Belgium) was used for rehydration experiments. The MNV, strain MNV-1.CW3 (kindly provided by Dr. Herbert Virgin, Washington University, St. Louis, USA) was propagated in RAW 264.7 cells (ATCC, Barcelona, Spain) grown in DMEM (Life Technologies, Gent, Belgium) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 20 mM HEPES, 0.075 g/L sodium bicarbonate, 1 mM sodium pyruvate, 100 U penicillin/mL, 100 μg/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. Virus stocks were generated from their seventh passage in cell culture and viral titres were determined by endpoint titration. Cell lines were regularly tested for Mycoplasma contamination.

2.2. Effect of post-exposure treatment with 2CMC in a MNV mouse model

AG129 mice (129/Sv mice deficient in IFN-α/β and IFN-γ receptors) originally from BK Universal, UK, were bred and housed at the Rega Institute under specific-pathogen-free conditions. All experiments were performed under the guidelines and authorization of the Ethical Committee of the KU Leuven (P101/2012).

For all experiments, age- and sex-matched mice, 8–12 weeks of age were randomly distributed per groups and infected by oral gavage with 6×10^4 $CCID_{50}$ (50% cell culture infectious dose) of MNV. Treatment with 2CMC was initiated either 1 h before infection (n = 6), 12 h (n = 10), 24 h (n = 10), 48 h (n = 9) or 72 h (n = 3) post-infection (pi) with a dose of 100 mg/kg/day, divided in two daily treatments (2 × 50 mg/kg) until day 7 pi, by the subcutaneous route. Saline was administered to untreated control mice following the same schedule as for 2CMC (n = 6). Treated and untreated mice were kept separate in independently ventilated cages for all the experiments. Bedding was replaced once a week. Starting at day 0 pi, mice were weighed daily and stools were collected from each animal (except for the 72 h pi group) and scored for consistency (0, normal feces; 1, mixed stool samples containing both solid and pasty feces; 2, pasty feces; 3, semiliquid feces; 4, liquid feces). When animals were severely ill (weight loss of >20% from the start of the experiment or >15% in 2 days, lethargy, watery squinted eyes) they were humanely euthanized using pentobarbital (Nembutal®). Blood was collected from the tail vein either (i) before infection or (ii) at one time point after infection or by cardiac puncture (iii) one week after re-challenge.

2.3. Effect of rehydration in 2CMC-treated and untreated MNV-infected mice

To study whether a combination of 2CMC-treatment and rehydration could have a beneficial effect and impact on the survival of MNV-infected animals, groups of 4 mice were treated with: (i) 2CMC 100 mg/kg/day starting 48 h pi (n = 4), (ii) LRS, for rehydration (n = 4) or (iii) a combination of 2CMC 100 mg/kg/day starting 48 h pi and LRS (n = 4). To determine when to start the rehydration of a particular animal, the fluid homeostasis of each mouse was evaluated daily by scoring the loss of skin turgor and weight loss. When mice were clinically dehydrated, i.e. had ≥5% body weight loss and/or loss of skin turgor, a volume equivalent to ~5% body weight of LRS at 37 °C was administered subcutaneously (plus animals were allowed to drink water *ad libitum*) according to the standard recommendations (Devey, 2010; Gargiulo et al., 2012). Animals were monitored once daily for symptoms, weight variation, loss of skin turgor and stool samples were collected for quantification of viral RNA.

2.4. Study of MNV replication in the small intestine of mice

AG129 mice (n = 18) were infected by oral gavage with 6×10^4 $CCID_{50}$ of MNV. At 0, 8, 12, 24, 48 and 72 h pi (n = 3 per time point), mice were euthanized for dissection of the small intestine which was processed through the Swiss roll technique (Moolenbeek and Ruitenberg, 1981) and fixed in 4% formaldehyde. For histological examination, 5 μm-thick intestinal tissue slides were embedded in paraffin, sectioned and stained with hematoxylin–eosin (H&E) or with a 1:5000 dilution of anti-MNV VLP (strain S99) rabbit polyclonal antibody or the corresponding pre-bleed rabbit serum (Taube et al., 2013).

The H&E stained slides of small intestine where the Peyer's Patches were present, were scored for the presence of MNV antigen as well as for the presence of inflammation and increased apoptosis of epithelial cells.

2.5. Re-challenge with MNV of 2CMC-treated infected mice

AG129 mice, which had been infected with MNV and treated with 2CMC starting 1 h before or at 12 h post infection, were re-challenged with MNV 3 (n = 7), 6 (n = 7) or 10 (n = 2) weeks after the first contact with the virus. During the week after re-challenge, mice were weighed daily and their general condition was assessed. Blood was collected from animals before re-challenge (at 3 weeks pi) and one week after re-challenge (week 7 pi and 11 pi).

2.6. Detection/quantification of specific anti-MNV IgG by ELISA

For detection of IgG in the blood of 2CMC-treated re-challenged mice, the Recombivirus™ Mouse Anti-Norovirus (MNV-1/VP1) IgG kit (Alpha Diagnostics/Gentaur, Belgium) was used according to the