Veterinary Microbiology 176 (2015) 370–377

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Short Communication

Antiviral effect of mefloquine on feline calicivirus *in vitro*

Phillip McDonagh [a], Paul A. Sheehy [b,1], Anne Fawcett [c,2], Jacqueline M. Norris [a,*]

[a] Faculty of Veterinary Science, Building B14, The University of Sydney, Sydney, NSW 2006, Australia
[b] Faculty of Veterinary Science, Building B19, The University of Sydney, Sydney, NSW 2006, Australia
[c] Faculty of Veterinary Science, Building B03, The University of Sydney, Sydney, NSW 2006, Australia

ARTICLE INFO

Article history:
Received 9 December 2014
Received in revised form 4 February 2015
Accepted 5 February 2015

Keywords:
Feline calicivirus
Antiviral
Cats
Mefloquine

ABSTRACT

Feline calicivirus (FCV) is an important viral pathogen of domestic cats causing clinical signs ranging from mild to severe oral ulceration or upper respiratory tract disease through to a severe fatal systemic disease. Current therapeutic options are limited, with no direct acting antivirals available for treatment. This study screened a panel of 19 compounds for potential antiviral activity against FCV strain F9 and recent field isolates *in vitro*. Using a resazurin-based cytopathic effect (CPE) inhibition assay, mefloquine demonstrated a marked inhibitory effect on FCV induced CPE, albeit with a relatively low selectivity index. Orthogonal assays confirmed inhibition of CPE was associated with a significant reduction in viral replication. Mefloquine exhibited a strong inhibitory effect against a panel of seven recent FCV isolates from Australia, with calculated IC50 values for the field isolates approximately 50% lower than against the reference strain FCV F9. *In vitro* combination therapy with recombinant feline interferon-ω, a biological response modifier currently registered for the treatment of FCV, demonstrated additive effects with a concurrent reduction in the IC50 of mefloquine. These results are the first report of antiviral effects of mefloquine against a calicivirus and support further *in vitro* and *in vivo* evaluation of this compound as an antiviral therapeutic for FCV.

1. Introduction

In domestic cats, feline calicivirus (FCV) is a common and important pathogen (Radford et al., 2007) typically resulting in upper respiratory tract signs or ulcerative oral lesions. Less frequent disease manifestations include a lameness syndrome, pneumonia, feline chronic gingivostomatitis syndrome (FCGS) (Lyon, 2005) and more recently the recognition of FCV-associated virulent systemic disease (FCV-VSD), a condition associated with high morbidity and mortality, even in vaccinated adult cats (Hurley and Sykes, 2003; Pedersen et al., 2000).

Both inactivated and modified live FCV vaccines are available to help control calicivirus-related disease in cats. They are generally effective at reducing the severity and duration of clinical signs, but do not prevent infection or shedding (Radford et al., 2007). The high level of antigenic variability amongst FCV isolates has raised concerns regarding the level of cross protection afforded by older vaccines (Radford et al., 2006), and the apparent limited protection afforded against isolates associated with FCV-VSD (Hurley and Sykes, 2003). Given these concerns and the significant impact of the virus in some cats, a safe and effective antiviral therapeutic would significantly advance feline medicine.

* Corresponding author. Tel.: +61 2 93517095.
E-mail addresses: phillip.mcdonagh@sydney.edu.au (P. McDonagh), paul.sheehy@sydney.edu.au (P.A. Sheehy), anne.fawcett@sydney.edu.au (A. Fawcett), jacqui.norris@sydney.edu.au (J.M. Norris).
[1] Tel.: +61 2 93515983.
[2] Tel.: +61 2 93512454.

http://dx.doi.org/10.1016/j.vetmic.2015.02.007
0378-1135/

There are currently no direct acting antiviral drugs registered for the treatment of FCV, although the immune modulating drug recombinant feline interferon-ω (rFeIFN-ω), which likely has indirect antiviral properties, has a registered indication for FCV treatment. Efficacy of both human and feline interferons has been demonstrated *in vitro* against FCV (Fulton and Burge, 1985; Mochizuki et al., 1994; Taira et al., 2005; Truyen et al., 2002) and the use of rFeIFN-ω has been reported to have a positive therapeutic effect in FCV infected cats in experimental and field efficacy trials (Ninomiya et al., 1991; Ohe et al., 2008). Treatment with rFeIFN-ω was also associated with an improvement in clinical signs in cats with refractory FCGS in a double-blinded placebo-controlled study, however FCV shedding was not monitored, making it unclear whether the improvement was due to the antiviral or immunomodulatory properties of interferon (Hennet et al., 2011). Recently, feline calicivirus specific antiviral phosphorodiamidate morpholino oligomers (PMO) were tested in naturally occurring outbreaks of FCV-VSD (Smith et al., 2008) and were highly efficacious, with treatment resulting in improved survival, reduction in shedding, and a more rapid clinical recovery.

The current study screened a panel of compounds for antiviral activity against FCV using a resazurin-based CPE inhibition assay. The antiviral effects of compounds identified during screening were confirmed with plaque reduction and virus yield reduction assays. Effective compounds were tested against a panel of recent FCV field isolates from Australia to confirm their effectiveness against more clinically relevant viruses. Effective compounds were also tested in combination with rFeIFN-ω, currently the only licenced treatment for FCV in Australia.

2. Materials and methods

2.1. Cell culture and viruses

Crandell Rees feline kidney (CRFK) cell line was propagated as outlined previously (McDonagh et al., 2014). The reference feline calicivirus strain F9 was kindly provided by Professor Gilkerson (University of Melbourne). Field isolates of FCV were collected from cats in Sydney and Melbourne and grown on CRFK cells. To confirm FCV as the causative agent of CPE from clinical samples, an indirect immunofluorescence assay was performed using an anti-FCV monoclonal antibody (clone S1-9; Custom Monoclonals International, Sacramento, USA). All viruses were titrated by a carboxymethylcellulose plaque assay on CRFK cells and stored in single use aliquots at −80 °C.

2.2. Test compounds

Nineteen compounds were selected based on reported *in vitro* antiviral effects against other RNA viruses (McDonagh et al., 2014). To determine an appropriate screening concentration the cytotoxicity of compounds was investigated using sequential resazurin and sulforhodamine B assays as previously reported (McDonagh et al., 2014). Test compound concentrations selected for subsequent antiviral screening were those resulting in cell viability of 80% or greater and are shown in Table 1. Stock solutions were prepared as outlined previously (McDonagh et al., 2014). The maximum final in-well DMSO concentration used in these studies was 0.33%, which was demonstrated to have no significant effect on cell viability or viral replication (data not shown).

2.3. Antiviral screening using CPE inhibition assay

Antiviral screening was performed using a modification of the optimised resazurin-based CPE inhibition assay outlined previously (McDonagh et al., 2014). After 1 h of compound exposure cells were infected with FCV strain F9 at MOI 0.01 (20 μl well^{-1}) for an infection period of 48 h with 50 μl of 1:10 dilution of 4 × stock resazurin in DMEM (final well concentration of resazurin 44 nM) added for the final 3.5 h. The duration of compound exposure in this assay was from 1 h prior to infection through to the assay endpoint, thereby allowing the identification of agents acting at any stage of the viral lifecycle. Plates were removed from the incubator for the final 30 min to allow the plates and media to equilibrate to room temperature. Fluorescent signals were measured with a FLUOstar Omega microplate reader (BMG Labtech, Mornington, VIC, Australia) using a 544 nm excitation filter and 590 nm emission filter with 8 flashes per well in bottom reading mode. Each treatment was performed in triplicate and results represent Mean ± SE of three independent experiments. The percentage inhibition of CPE was calculated by:

$$\text{CPE inhibition}(\%) = \frac{\text{RFU}_{\text{TX}} - \text{RFU}_{\text{V}(+)}}{\text{RFU}_{\text{V}(-)} - \text{RFU}_{\text{V}(+)}} \times 100$$

where RFU_{Tx} is the mean fluorescence intensity of treated infected cells; $\text{RFU}_{\text{V}(+)}$ is the mean fluorescence intensity in untreated infected cells; and $\text{RFU}_{\text{V}(-)}$ is the mean

Table 1
Compounds selected for antiviral screening.

Compound	Screening concentration
Chloroquine diphosphate[a]	25 μM
Quercetin[a]	10 μM
Curcumin[b]	10 μM
Rutin trihydrate[b]	25 μM
Indomethacin[b]	10 μM
Glycyrrhizic acid[a]	25 μM
Hesperidin[b]	50 μM
Aurintricarboxylic acid[a]	2.5 μM
Hesperetin[b]	50 μM
Mefloquine hydrochloride[a]	10 μM
Artesunate[a]	1 μM
Ribavirin[a]	2.5 μM
Baicalin hydrate[b]	10 μM
Hexamethylene amiloride[b]	10 μM
Cinanserin[b]	20 μM
Artemisinin[a]	25 μM
Niclosamide[b]	0.25 μM
Lactoferrin[a]	0.5 mg ml^{-1}
Recombinant feline interferon ω[c]	100 units ml^{-1}

Superscripts indicate compound supplier.
[a] Sigma–Aldrich.
[b] Santa Cruz Biotechnology.
[c] Virbac.