EFFECTS OF SOME PICORNAVIRUS INHIBITORS ON THE REPLICATION OF FELINE CALICIVIRUS FCV IN CRFK CELLS

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ABSTRACT

Obtaining of substances suppressing replication of caliciviruses is of special interest due to their particular role in the veterinary and human infectious pathology. Investigations for development of effective anti-calicivirus chemotherapy are important due to lack of specific means for calicivirus infections treatment. Caliciviridae possess a RNA(+) genome and similar structure as Picornaviridae, but reveal a different genome strategy. In a view of some similarities, a screening for anti-calicivirus activity of several highly efficient inhibitors of picornavirus replication was carried out. Research was carried out with Feline calicivirus (FCV), Crandell’s Feline Kidney cell line (CrFK) and the following compounds: Arildone, Disoxaril, S-7, Guanidine hydrochloride, PTU-23, HBB, Ribavirin and Oxoglucine. Anti-calicivirus activity and citotoxicity were tested through CPE inhibition test and neutral red uptake assay (vs. virus inoculating doses ranging within 1 and 10 000 CCID₅₀). Significant effects of HBB, PTU-23, Ribavirin and Oxoglucine and a slight activity of S-7 were indicated, while Arildone, Disoxaril and Guanidine hydrochloride did not show influence.

Keywords: caliciviruses, noroviruses, feline calicivirus, anti-calicivirus chemotherapy, antivirals.

INTRODUCTION

Caliciviruses are important pathogens of man and animals. Family Caliciviridae is divided into four genera – Norovirus, Sapporovirus, Lagovirus and Vesivirus [2,14,21]. Noroviruses (strains Lordsdale, Mexico, Hawaii, Snow Mountain, Desert Shield and Southampton) and Sapporoviruses (strains London/29845, Manchester, Houston/86, Houston/90, Sapporo/82 and Parkville) as human pathogens are among the leading ethiological agents of acute viral gastroenteritis in people of all ages in industrialized countries, where they may be responsible for 68–80% of all outbreaks of viral gastroenteritides [1,14,15,20,21]. Members of animal Caliciviruses (genus Lagovirus and Vesivirus) cause a variety of host-dependent diseases: Feline Calicivirus (FCV) – acute upper respiratory disease and stomatitis in cats [22]; Vesicular Exanthema of Swine Virus (VESV) – vesicular exanthema in swine; San-Miguel Sea Lion Virus (SMSV) – vesicular lessions and abortions in some marine mammals (sea lions, seals, dolphins etc.); Rabbit Haemorrhagic Disease Virus (RHDV) - rabbit haemorrhagic disease; European Brown Hare Syndrome Virus (EBHSV) - European Brown Hare Syndrome in rabbits [2].

Caliciviridae are nonenveloped viruses. The virions are 35-40 nm in diameter and have icosahedral symmetry and a distinctive morphology – a series of cup-like surface depressions are viewed by negative stain electron microscopy. Caliciviruses possess a single-stranded (+) RNA genome, which is 7 – 8 kb in length and is polyadenylated [3,4,7,8,16]. The investigations for development of effective anti-calicivirus chemotherapy and obtaining of substances suppressing the replication of caliciviruses (noroviruses respectively) are important due to: their role in the human infectious pathology (as known the noroviruses are serious problem of public health in European countries and the USA); their role in the veterinary infectious pathology (some diseases as rabbit haemorrhagic disease cause high mortality and economic damages); lack of specific
means for treatment and prevention (in humans) [6,17,20].

MATERIALS AND METHODS

Materials

Cells. Our study was carried out on Crandell's Feline Kidney cell line – CrFK (Centre for Research on Environmental Microbiology - CREM, Faculty of Medicine, University of Ottawa, Canada), which was cultivated in monolayer in DMEM supplemented with 10% FBS (Gibco), at 37°C in a humidified 5% CO2 atmosphere for 24 hours [1].

Virus. Feline Calicivirus FCV, F9 strain (CREM, Faculty of Medicine, University of Ottawa, Canada) as one of the few cultivatable members of Caliciviridae [18] and the best available surrogate for Norovirus [1,5] was used.

Antivirals. In a view of the similarities between Caliciviridae and Picornaviridae [5], investigations for anti-calicivirus activity of eight highly efficient inhibitors of Picornavirus replication were carried out. Used Picornavirus replication inhibitors belong to the following groups: inhibitors of early stages of virus replication cycle or WIN compounds - Disoxaril (WIN 51711; 5-[7-[4(4,5-dihydro-2-oxazolil)phenoxy]heptil]-3-methyl-izoxazole) [9], Arildone (WIN 3802; 4-[6-(2-chloro-4-methoxyphenoxy)hexyl]3,5-heptadion) [9]; Methylthiopyrimidine (S-7) [9]; inhibitors of virus-specific RNA synthesis - Guanidine hydrochloride [9,13], PTU-23 (N-phenyl-N’-2-hydroxyphenylthiourea) [9] and benzimidazoles – HBB (2-α-hydroxybenzyl benzimidazole) [9,13]; compounds possessing other mechanism of action - Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) [9,10,12,19] and Oxoglaucine [9].

Methods

Anti-calicivirus activity was tested by CPE Inhibition Test in monolayer cell culture in a 96-well cell culture plate (Cellstar, Greiner bio-one) vs. virus inoculation doses ranging within 1 and 10 000 CCID50 and Neutral Red Uptake Assay in a 96-well cell culture plate (Cellstar, Greiner bio-one) and measuring of the absorption at λ = 540 nm in a ELISA-reader to determine the individual cytotoxicity and antiviral effect of all compounds was used. NRU test is a specific cell survival / viability chemosensitivity assay based on the ability of viable cells to incorporate intracellularly and bind a supravital dye Neutral Red (NR Fluka). Damaged or dead cells as a result of action of xenobiotics or virus could not take up the NR [11].

RESULTS

Figure 1. (HBB)

Figure 1. Antiviral (CPE inhibitory) effect of HBB on the replication of FCV-F9 in CrFK cells. Virus inoculation dose: (A) 1000 CCID50; (B) 100 CCID50; (C) 10 CCID50. In picornaviruses HBB inhibits the activity of virus-specific RNA-polymerase, resulting in a selective suppression of the ssRNA synthesis (dsRNA synthesis remaining intact).
Figure 2. (PTU-23)

Figure 2. Antiviral (CPE inhibitory) effect of PTU-23 on the replication of FCV-F9 in CrFK cells. Virus inoculation dose: (A) 1000 CCID_{50}, (B) 100 CCID_{50}, (C) 10 CCID_{50}. In picornaviruses PTU-23 inhibits the synthesis of viral RNA, a result of suppression of the synthesis of a viral protein with a regulatory function in the replication cycle.

Figure 3. (Ribavirin)

Figure 3. Antiviral (CPE inhibitory) effect of Ribavirin on the replication of FCV-F9 in CrFK cells. Virus inoculation dose: (A) 1000 CCID_{50}, (B) 100 CCID_{50}, (C) 10 CCID_{50}. The broad-spectrum antiviral agent Ribavirin exerting polycomponent mechanism of action, based predominantly of various effects on the hostcell (reduction of GTP pool, inhibition of 5' cap formation on mRNAs)

Figure 4. (Oxoglaucine)

Figure 4. Antiviral (CPE inhibitory) effect of Oxoglaucine on the replication of FCV-F9 in CrFK cells. Virus inoculation dose: (A) 1000 CCID_{50}, (B) 100 CCID_{50}, (C) 10 CCID_{50}. Oxoglaucine is a recently described aporphine alkaloid (isolated from the aerial parts of the plant Glaucum flavum Cranz), possessing a large-spectrum anti-enteroviral effect.
Figure 5. (S-7)

Antiviral (CPE inhibitory) effect of S-7 on the replication of FCV-F9 in CrFK cells. Virus inoculation dose: (A) 1000 CCID$_{50}$; (B) 100 CCID$_{50}$; (C) 10 CCID$_{50}$. S-7 interacting directly with picornavirus particles, they become stabilized, thus inhibiting the virus uncoating.

Figure 6. (Guanidine hydrochloride)

Antiviral (CPE inhibitory) effect of Guanidine hydrochloride on the replication of FCV-F9 in CrFK cells. Virus inoculation dose: (A) 1000 CCID$_{50}$; (B) 100 CCID$_{50}$; (C) 10 CCID$_{50}$. Guanidine HCl interacting with virus 2C protein in picornaviruses and specifically blocks the initiation of RNA(-) synthesis.

Figure 7. (Disoxaril)

Antiviral (CPE inhibitory) effect of Disoxaril on the replication of FCV-F9 in CrFK cells. Virus inoculation dose: (A) 1000 CCID$_{50}$; (B) 100 CCID$_{50}$; (C) 10 CCID$_{50}$. Molecules of WIN compounds stabilizing virus particles and inhibit their uncoating by direct insertion into the hydrophobic canyon (within the VP1) in picornaviruses.

Figure 8. (Arildone)

Antiviral (CPE inhibitory) effect of Arildone on the replication of FCV-F9 in CrFK cells. Virus inoculation dose: (A) 1000 CCID$_{50}$; (B) 100 CCID$_{50}$; (C) 10 CCID$_{50}$. Molecules of WIN compounds stabilizing virus particles and inhibit their uncoating by direct insertion into the hydrophobic canyon (within the VP1) in picornaviruses.
CONCLUSIONS
- a significant anti-calicivirus activity possess compounds known as inhibitors of virus-specific RNA synthesis - HBB (320, 200 and 100 M) and PTU-23 (320, 200, 160 and 100 M). The maximal tolerated concentration (MTC) for these two compounds is 320 M.
- the broad-spectrum antiviral agent Ribavirin (32, 16 and 10 M) and a recently described aporphine alkaloid Oxoglaucine (3.2, 1, 0.32 and 0.1 M) (isolated from the aerial parts of the plant Glaucum flavum Cranz), also present anti-calicivirus activity. MTC is 32 M for Ribavirin; 10 M for Oxoglaucine.
- a slight effect against FCV of compound S-7 (100 M) was indicated. The MTC for S-7 is 320 M.
- compounds interacting with hydrophobic canyone in picornaviruses - Disoxaril and Arildone (WIN-compounds) and compound Guanidine HCl did not show an influence.

REFERENCES
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