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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 Oxoglaucine. 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 $CCID_{50}$). Significant effects of HBB, PTU-23, Ribavirin and Oxoglaucine 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% CO_2 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 benzimidasoles – HBB (2-α-hydroxybenzyl benzimidasole) [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 $CCID_{50}$ 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)

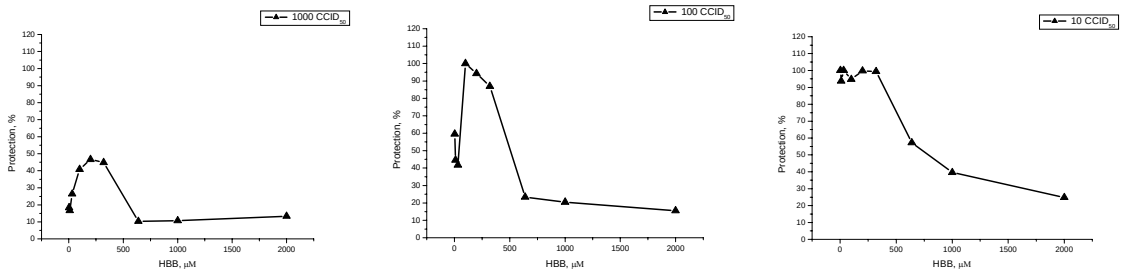
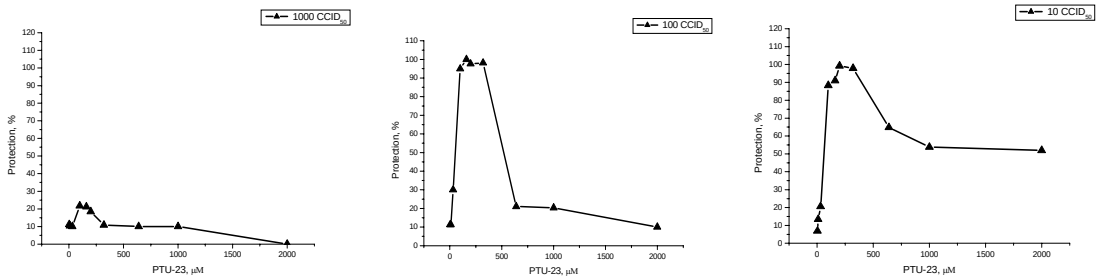


Figure 1. *Antiviral (CPE inhibitory) effect of **HBB** on the replication of FCV-F9 in CrFK cells. Virus inoculation dose: **(A)** 1000 $CCID_{50}$; **(B)** 100 $CCID_{50}$; **(C)** 10 $CCID_{50}$.*
In picornaviruses HBB inhibits the activity of virus-specific RNA-polymerase, resulting in a selective suppression of the ssRNA synthesis (dsRNA synthesis remaining intact).

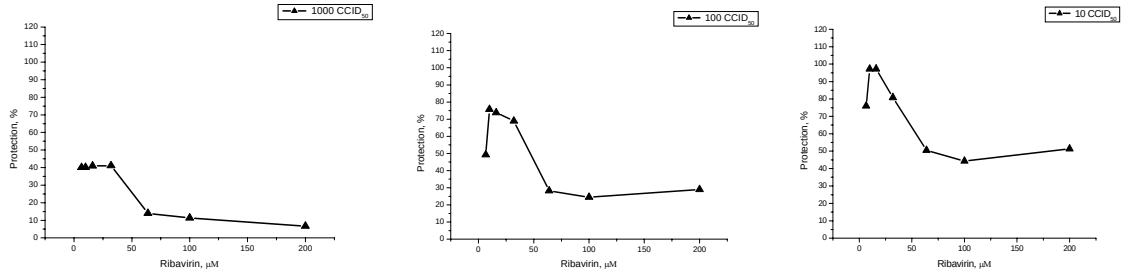
Figure 2. (PTU-23)



(A) (B) (C)

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.

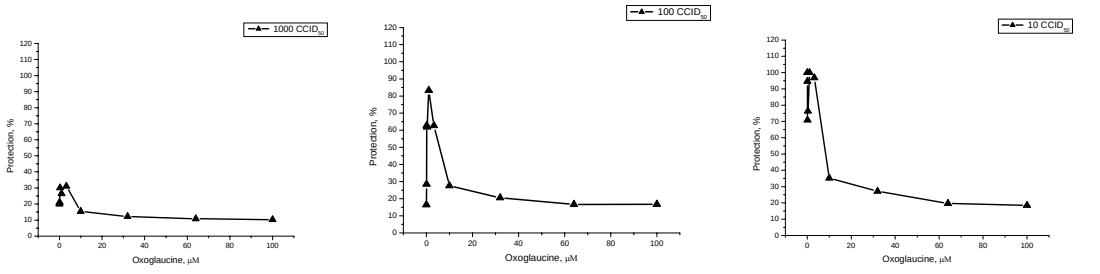
Figure 3. (Ribavirin)



(A) (B) (C)

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 host cell (reduction of GTP pool, inhibition of 5'-cap formation on mRNAs

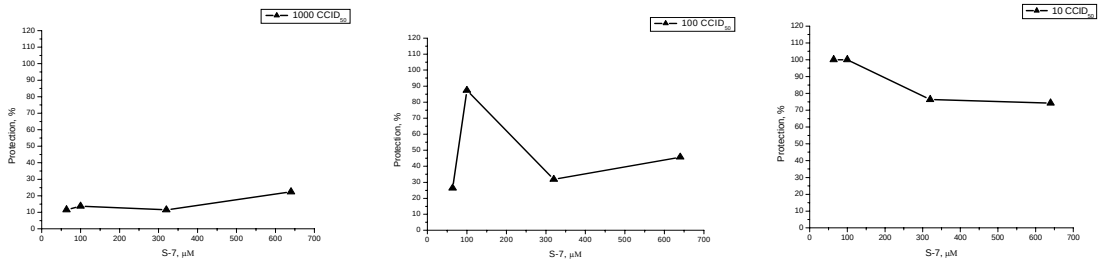
Figure 4. (Oxoglaucine)



(A) (B) (C)

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

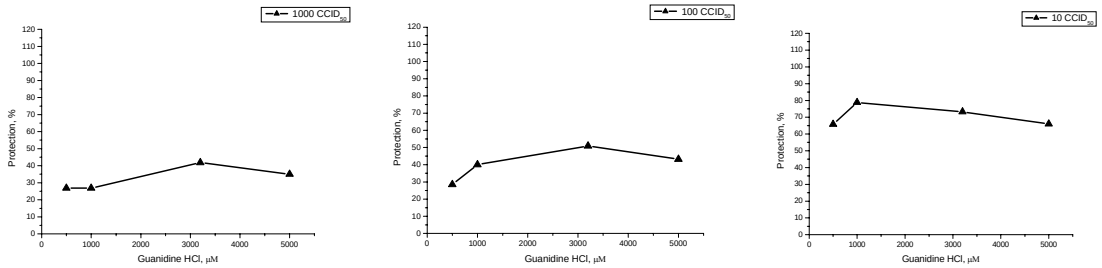
Figure 5. (S-7)



(A) (B) (C)

Figure 5. *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.

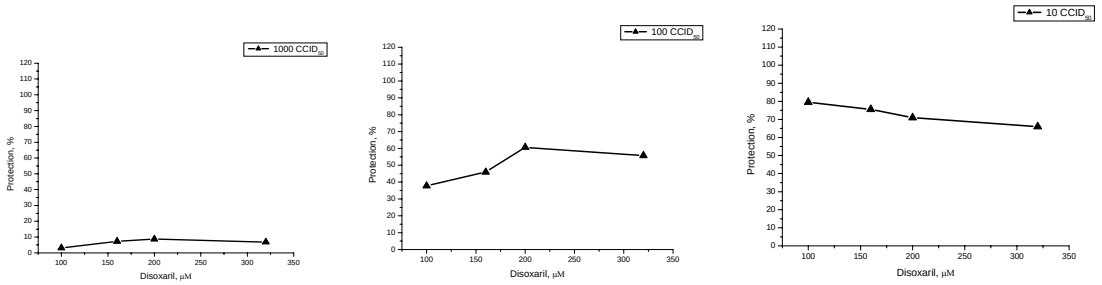
Figure 6. (Guanidine hydrochloride)



(A) (B) (C)

Figure 6. *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.*

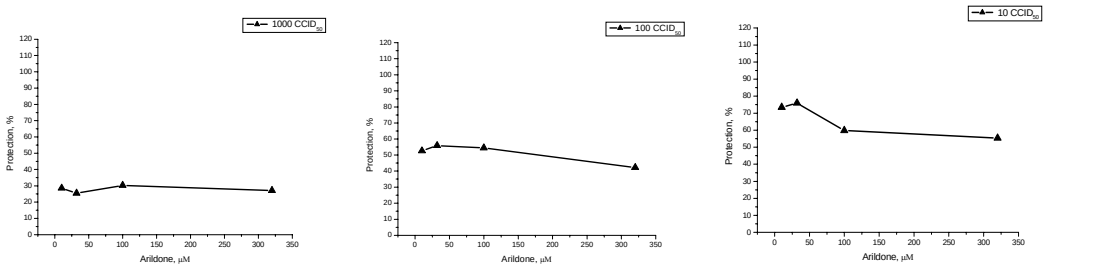
Figure 7. (Disoxaril)



(A) (B) (C)

Figure 7. *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 $CCID_{50}$.*

Figure 8. (Arildone)



(A) (B) (C)

Figure 8. *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

1. Bidawid S., Malik N., Adegbunrin O., Satar S.A., Farber J.M. (2003): A feline kidney cell line - based plaque assay for feline calicivirus, a surogate for Norwalk virus. Journal of Virological Methods, 107; 163 - 167.
2. Green K.Y., Ando T., Balayan M.S., Berke T., Clarke I.N., Estes M.K., Matson D.O., Nakata S., Neill J.D., Studdert M.J., Thiel H.J. (2000):Taxonomy of the caliciviruses. Journal of Infectious Diseases, 181; 322 - 330.
3. Herbert T.P., Brierley I., Brown T.D. (1997): Identification of a protein linked to the genomic and subgenomic mRNA of feline calicivirus and its role in translation. Journal of General Virology, Vol. 78; 1033 - 1040.
4. Clarke I. N. and Lambden P.R. (1997): The molecular biology of caliciviruses. Journal of General Virology, 78**;** 291 - 301.
5. Nuanualsuwan S.and Cliver D.O. (2003): Capsid functions of inactivated Human Picornaviruses and Feline calicivirus. Applied and Environmental Microbiology, Vol. 69, No. 1; 350 - 357.
6. Brumley D. (2003): Presentation to Board of Public Health, Duxbury, Massachusetts. Sources: Centers for Disease Control; Merck Manual.
7. Willcocks M.M., Carter M.J., Roberts L.O. (2004): Cleavage of eukaryotic initiation factor eIF4G and inhibition on host - cell protein synthesis during Feline calicivirus infection. Journal of General Virology, 85; 1125 - 1130.
8. Asanaka M., Atmar R.L., Ruvolo V., Crawford S.E., Neill F.H., Estes M.K. (2005): Replication and packaging of Norwalk virus RNA in cultured mammalian cells. PNAS Journal, Vol 102, No 29; 10327 - 10332.
9. Galabov A.S., Angelova A. (2006): Antiviral Agents in the Prevention and Treatment of Virus-Induced Diabetes. Anti-Infective Agents in Medicinal Chemistry, No 5, 293 – 307.
10. Povey, R.C. (1978): In vitro antiviral efficacy of ribavirin against feline calicivirus, feline viral rhinotracheitis virus, and canine parainfluenza virus, American Journal of Veterinary Research, 39 (1), 175-178.
11. Player M.R., Barnard D.L., Torrence P.F. (1998): Potent inhibition of respiratory sincytial virus replication using a 2-5 A antisense chimera targeted to signals within the virus genomic RNA. Medical Sciences, Proc. Natl. Acad. Sci. USA, vol. 95, 8874 – 8879.
12. Bean B. (1992): Antiviral Therapy: current concepts and practices. Clinical Microbiology Reviews, vol. 5, No 2, 146 - 182.
13. Shimizu H., Agoh M., Yoshida H., Yoshii K., Yoneyama T., Hagiwara A., Miyamura T. (2000): Mutations in the 2C Region of Poliovirus Responsible for Altered Sensitivity to Benzimidazole Derivates. Journal of Virology, vol. 74, No 9, 4146 – 4154.
14. Gerba C.P., Kayed D. (2003): Caliciviruses: A major cause of foodborne illness. Journal of Food Science, Vol. 68, No 4, 1136 - 1142.
15. Rockx, B., de Wit M., Vennema H., Vinje J., de Bruin E., van Duynhoven Y., and Koopmans M. (2002): Natural history of human calicivirus infection: a prospective cohort study.

Clinical Infectious Diseases, 35**;** 246 - 253.
16. Sosnovtsev S.V., Belliot G., Chang K.O., Onwudiwe O., Green K.Y. (2005): Feline Calicivirus VP2 is essential for the production of infectious virions. Journal of Virology, Vol. 79, 4012 – 4024.
17. Christensen M.L. (1989): Human viral gastroenteritis. Clinical Microbiology Reviews, vol. 2, No 1, 51 – 89.
18. Stuart A.D., David T., Brown K. (2006): Entry of Feline Calicivirus is dependent on Clathrin – Mediated Endocytosis and Acidification in endosomes. Journal of Virology, Vol. 80, No 15, 7500 – 7509.
19. Browne, M.J. (1979): Mechanism and Specificity of Action of Ribavirin. Antimicrobial agents and Chemotherapy, vol. 15, No 6, 747 – 753.
20. Feng X. and Jiang X. (2007): Library Screen for Inhibitors Targeting Norovirus Binding to Histo-Blood Group Antigen Receptors. Antimicrobial agents and Chemotherapy, vol. 51, No 1, 324– 331.
21. Atmar R.L., Estes M.K. (2001): Diagnosis of Noncultivatable Gastroenteritis Viruses, the Human Caliciviruses. Clinical Microbiology Reviews, vol. 14; 15-37.
22. Radford A.D., Coyne K.P., Dawson S., Porter C.J., Gaskell R.M. (2007): Feline Calicivirus. Veterinary Research, Vol. 38, 319 – 335.