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A preclinical assessment to repurpose drugs to target type 1 diabetes associated type B Coxsackieviruses.

Running title: Antivirals targeting type 1 diabetes associated type B Coxsackieviruses

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Conflicts of interest

Heikki Hyöty is a minor (<5%) shareholder and the chairman of the board of Vactech Ltd., which develops vaccines against picornaviruses. No other potential conflicts of interest relevant to this article are reported.

What's new?

- Type B coxsackieviruses are associated with type 1 diabetes.
- Although many drugs have shown anti-enterovirus properties, no antiviral drug has currently been licensed specifically for the treatment of enterovirus infections.
- Repurposing antiviral drugs could be an attractive opportunity in type 1 diabetes prevention trials.
- Dose and spectrum tests against type B Coxsackieviruses revealed Hizentra, Enviroxime, Ribavirin, Pleconaril, and Favipiravir would be the best candidates for this strategy.
- Ribavirin and Itraconazole were less effective but were able to impair virus replication.
- Our results can be used to design clinical trials aimed at eradication of persistent infection from the pancreas and preventing/treating type 1 diabetes.
- Using antiviral drugs to treat children with type 1 diabetes will help to elucidate the involvement of enteroviruses, including coxsackieviruses, over the course of the condition.

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Abstract

Aims

Type 1 diabetes has been linked to enterovirus infections, specifically type B Coxsackieviruses; possibly through a persistent infection in beta cells. To test this hypothesis, antiviral drug treatment is currently being evaluated in a clinical trial, in children with newly diagnosed type 1 diabetes. No antiviral drug has been licensed specifically for the treatment of enterovirus infections. Therefore, repurposing licensed drugs offers an attractive option for facilitating further clinical trials. We systematically screened several antiviral drugs for their efficacy against type B Coxsackieviruses.

Methods

10 drugs with different antiviral mechanisms were analysed for their efficacy against prototype strains of type B Coxsackieviruses in A549 cells. Cell viability was quantified in fixed cells using a colorimetric assay. Median effective dose was interpolated from the triplicated experiments and the dose response curves were generated for each drug-virus combination. Drug cytotoxicity was similarly quantified and selectivity indices calculated.

Results

Hizentra, Pleconaril, Fluoxetine, Norfluoxetine, Ribavirin, Favipiravir, and Guanidine-Hydrochloride were able to abrogate infection by all tested viruses, with the exception of complete inefficacy of Pleconaril against Coxsackievirus B3 and Favipiravir against Coxsackievirus B2. The effective dose for Hizentra, Enviroxime, Ribavirin and Pleconaril were clearly below their therapeutic serum concentrations, while the effective concentration of Fluoxetin, Norfluoxetine and Itraconazole exceeded their therapeutic serum concentrations. Lovastatin and Azithromycin did not efficiently block type B Coxsackieviruses.

Conclusion

Hizentra, Enviroxime, Pleconaril, Ribavirin, and Favipiravir are effective against type B Coxsackieviruses, *in vitro* in their therapeutic serum concentrations. These antiviral drugs are therefore attractive candidates for type 1 diabetes prevention/treatment trials. They can also be used in other clinical conditions caused by type B Coxsackieviruses.

Keywords

Antiviral drug, Azithromycin, type B Coxsackievirus, Enviroxime, Favipiravir, Fluoxetine, Itraconazole, Immunoglobulin, Lovastatin, Pleconaril, Ribavirin, Repurposing drugs, Type 1 diabetes

Introduction

Type B Coxsackieviruses (CVB) belong to the species Enterovirus-B within the family *Picornaviridae*, icosahedral non-enveloped RNA viruses with mRNA polarity. CVB include six members (1-6) causing e.g. common-cold, aseptic meningitis, herpangina, myocarditis, pericarditis, and multi-organ life-threatening infections in young infants. They constitute about 24% of all reported enteroviruses (EV) by the National Enterovirus Surveillance System (NESS) 1970-2018 [1]. They have also been linked to chronic dilated cardiomyopathy and chronic fatigue syndrome [2-4].

Type 1 diabetes has also been linked to CVB [5-7]. It has been suggested that a persistent infection in insulin-producing pancreatic beta cells could contribute to the development of type 1 diabetes [8, 9]. Therefore, those antiviral drugs that are effective against CVB could offer an option to prevent/treat type 1 diabetes by eradicating such infections. In fact, to assess this opportunity, newly-diagnosed type 1 diabetes patients are currently being recruited into the DiViDIntervention trial in Norway (EudraCT No. 2015 003350-1), a randomized controlled trial using antiviral drugs [10].

Currently, no antiviral drug has been licensed for the treatment of EV infections, as Pleconaril did not get the approval for treatment of the common cold. Nevertheless, Pleconaril does reduce the mortality in severe EV infections in young infants [11]. In addition, certain drugs that are prescribed for other indications have shown anti-EV activity *in vitro*, but the effect of these compounds against different members of CVB has not been systematically studied. Therefore, we set out to evaluate the possibility of repurposing clinically used drugs, which have shown activity against at least one EV strain *in vitro*, to block the infection of all prototype strains of CVB in cell culture. The aim was to identify drugs with broad anti-CVB activity, to facilitate their possible use in the treatment of severe and/or persistent CVB infections and in clinical trials evaluating the possible role of CVB in type 1 diabetes.

We selected drugs targeting different steps in the viral replication cycle, including Pleconaril (a pocket factor replacer and uncoating blocker of EV), Enviroxime (EV 3A blocker), Ribavirin (a nucleoside analogue), Hizentra (an immunoglobulin that contains neutralizing CVB antibodies), Favipiravir (an inhibitor of RNA-dependent RNA polymerases of RNA viruses), Fluoxetine (commercially known as Prozac) and its active metabolite Norfluoxetine (both potential EV 2C blockers), Itraconazole (EV 3A blocker through targeting cellular oxysterol-binding protein), Azithromycin (interferon and interferon-stimulated gene up-regulator), and Lovastatin (possible viral receptor down-regulator or disruptor of the membranes of viral replication organelles). Guanidine Hydrochloride (an EV 2C ligand) was used as a positive control.

Methods

Antiviral activity assay

Mycoplasma-free A549 (human lung carcinoma epithelial, ATCC) cells were used. Cells were maintained in Ham's F12 media (Sigma-Aldrich) with 10% Fetal Calf Serum (Gibco) and Penicillin-Streptomycin (Gibco) in a humidified chamber with 5% CO_2 at 37°C. Cell monolayers were infected in 96 well plates (Nunc) with 95% confluency in the absence of antibiotics with individual CVB (ATCC prototype strains, Wesel, Germany; supplementary Table 1) with different concentrations of drugs and 10,000 tissue culture infectivity dose 50% of each virus (quantified by Reed and Munch method). Viable plastic-bound cells were fixed-stained at 48 hours post infection using formaldehyde (0.5%)-crystal violate (0.05%) (Electron Microscopy Sciences, Oy FF-Chemicals Ab, Finland) for 20 minutes at room temperature. The intensity of the colour was identified by solubilizing the stained cells using 1% Sodium Dodecyl Sulphate in Phosphate Buffered Saline, for 10 minutes at room temperature and measuring the absorbance of each well at 595nm using an ELISA plate reader (Perkin Elmer Victor2V Multilabel Counter 1420-040). The cell viability (%) was calculated comparing the absorbance values of the test wells to those of mock-infected wells representing 100% cell viability.

Since Hizentra (human immunoglobulin) and Pleconaril interact directly with the virus to block the infectivity, they were incubated with the virus at 37°C for one hour prior to the infection and throughout the post infection period. As recommended (see supplementary Table 2), Azithromycin and Lovastatin were added to the cells 24 hours prior to infection and the other drugs were added on the cells one hour before virus infection (see supplementary Table 1). Control wells included no drug or virus. Drug cytotoxicity was also monitored by analysing cells exposed to the drug without any virus for 48 hours.

Identification and quantification of antiviral effect

Median effective dose (ED_{50}), that prevents 50% of cell death due to virus infection, was calculated from the triplicated experiments for each drug and individual viruses. ED_{50} directly demonstrates the efficiency of the drug against individual viruses. The graphs were generated using the viability data and concentrations of the drugs used in the study. The built-in log(agonist) vs. response statistics package, based on a nonlinear regression method in Graph Pad Prism program (version 5.02), was used to generate the graphs. In some cases, the ED_{50} could not be calculated since some drugs did not reach 50% blockage of the virus infection. Cytotoxic concentration 50% (CC_{50}, the concentration of the drug that kills half of the cells in uninfected samples) of each drug was also calculated in the absence of the viruses using a similar approach and different concentrations of each drug. The selectivity index ($SI = CC_{50}/ED_{50}$), representing the safety profile of the drug related to antiviral dose, was calculated for each drug (Table 1). The program was not able to calculate CC_{50} for Lovastatin. Therefore, the value of the highest Lovastatin concentration used in the assay was used in place of a CC_{50} value. For all drugs a conservative "minimum SI" is presented using the highest concentration of the ED_{50} against CVB. All background information and relevant references for virus strains and the drugs used in the study are reported in supplementary Table 1 and 2.

Results

Individual ED_{50} values for each drug-virus combination are reported in Table 1 and the dose response curves are reported in Fig. 1. Briefly, Hizentra, Pleconaril, Fluoxetine, Norfluoxetine, and Guanidine Hydrochloride were able to abrogate infection by all tested viruses, with the known exception that Pleconaril was not able to block the ATCC strain of CVB3 (Table 1). Ribavirin blocked all viruses but with less than 100% efficacy and was more cytotoxic than any other drug. Lovastatin blocked only CVB2 with lower efficacy than other drugs. Azithromycin only blocked CVB3.

The ED_{50} values (horizontal broken lines crossing the dose response curves in Fig. 1) for Hizentra, Enviroxime, Ribavirin and Pleconaril were clearly below the serum concentrations reached in clinical treatments (vertical lines in Fig. 1), suggesting that the antiviral effect could be reached by the doses recommended for their routine clinical indications. On the other hand, for Fluoxetine/Norfluoxetine and Itraconazole ED_{50} values were higher than the serum concentrations reached by recommended doses (up to 4.5, and 2 times higher, respectively). Lovastatin and Azithromycin performed poorly and did not even reach ED_{50} for many members of CVB. By contrast, Hizentra blocked the infection of all CVB even at low concentrations, therefore the ED_{50} value was not calculated as it was far below the recommended doses used in the clinic. Since Hizentra is a pooled antiserum, its antiviral activity is reached mainly by neutralizing antibodies. Therefore, lower reactivity to CVB6 reflects a generally lower titter of neutralizing antibodies to this virus, that could be due to a low prevalence of CVB6 in human population.

Conclusion

This study characterized the anti-CVB activity of drugs that had already been reported to block some members of EV but not exclusively tested for activity against infection by all members of CVB (proposed to be associated with type 1 diabetes). Hizentra, Enviroxime, Pleconaril, Ribavirin, and in many cases Favipiravir, were highly effective against CVB *in vitro* at the concentrations reached in serum at their recommended therapeutic doses (identified by other studies shown in Table 1). Therefore, it is possible that these drugs could provide clinical benefits in acute or persistent CVB infections. Further studies, particularly carefully

designed clinical trials, would be needed to explore this possibility among patients with type 1 diabetes. This has already started with the DIVIDintervention trial on patients newly diagnosed with type 1 diabetes [10]. This unique trial uses a combination of Pleconaril and Ribavirin which were both found to be effective against CVBs in the present study.

The advantage of the antiviral drugs we currently tested is that their safety profile is already characterized. For example, as well as potentially causing anaemia, Ribavirin is contraindicated during pregnancy and in the male partners of women planning pregnancy due to its teratogenic potential [12]. Fluoxetine is not licensed in children below the age of 7 years because of a greater risk of suicide during the first few months of treatment and it may also disturb glucose metabolism in diabetic people requiring adjustments to insulin dosage [12]. Fluoxetine may also induce QT prolongation when used together with e.g. Azithromycin or Itraconazole [12]. Lovastatin has been contraindicated for use with Itraconazole due to the substantial increased risk of developing myopathy, rhabdomyolysis, and acute renal failure [12]. Immunoglobulin (Hizentra) concentrates which have been used off-label for measles prophylaxis and for the prevention of poliomyelitis before the polio vaccine era, is known to be associated with Thromboembolism [12].

In conclusion, the present study identified several drugs (Hizentra, Enviroxime, Pleconaril, Ribavirin, and Favipiravir) that could be beneficial in the treatment of CVB infections and which could be useful in type 1 diabetes intervention trials.

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References

[1] CDC-USA (May 10, 2018) The National Enterovirus Surveillance System (NESS), USA. Available from: https://www.cdc.gov/surveillance/ness/pubs.html, accessed 06/06 2019

[2] Bowles NE, Bayston TA, Zhang HY, et al. (1993) Persistence of enterovirus RNA in muscle biopsy samples suggests that some cases of chronic fatigue syndrome result from a previous, inflammatory viral myopathy. J Med 24:145-160

[3] Klingel K, Hohenadl C, Canu A, et al. (1992) Ongoing enterovirus-induced myocarditis is associated with persistent heart muscle infection: quantitative analysis of virus replication, tissue damage, and inflammation. Proc Natl Acad Sci U S A 89:314-318

[4] Cunningham L, Bowles NE, Lane RJ, Dubowitz V, Archard LC (1990) Persistence of enteroviral RNA in chronic fatigue syndrome is associated with the abnormal production of equal amounts of positive and negative strands of enteroviral RNA. J Gen Virol 71 (Pt 6):1399-1402

[5] Sioofy-Khojine AB, Lehtonen J, Nurminen N, et al. (2018) Coxsackievirus B1 infections are associated with the initiation of insulin-driven autoimmunity that progresses to type 1 diabetes. Diabetologia 61:1193-1202

[6] Oikarinen S, Tauriainen S, Hober D, et al. (2014) Virus antibody survey in different European populations indicates risk association between coxsackievirus B1 and type 1 diabetes. Diabetes 63:655-662

[7] Laitinen OH, Honkanen H, Pakkanen O, et al. (2014) Coxsackievirus B1 is associated with induction of beta-cell autoimmunity that portends type 1 diabetes. Diabetes 63:446-455

[8] Krogvold L, Edwin B, Buanes T, et al. (2015) Detection of a low-grade enteroviral infection in the islets of langerhans of living patients newly diagnosed with type 1 diabetes. Diabetes 64:1682-1687

[9] Richardson SJ, Rodriguez-Calvo T, Gerling IC, et al. (2016) Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. Diabetologia 59:2448-2458

[10] EU Clinical Trials Register (EudraCT): European Medicines Agency © 1995-2018 | 30 Churchill Place, Canary Wharf, London E14 5EU. EudraCT Number: 2015-003350-41, The Diabetes Virus Detection and Intervention Trial (DiViDIntervention); 2017 Jan 25 [2018 Oct 15]; [1 page]. Available from: https://www.clinicaltrialsregister.eu/ctr-search/trial/2015-003350-41/NO.

[11] Rotbart HA, Webster AD, Pleconaril Treatment Registry Group (2001) Treatment of potentially life-threatening enterovirus infections with pleconaril. Clin Infect Dis 32:228-235

[12] ©2018 PDR L (2018) Drug Summary. Available from: https://www.pdr.net/browse-by-drug-name, accessed 11.11 2018

Table 1: Median effective dose (EE_{50}) values of each drug for individual viruses.
This table presents the ED_{50} and cytotoxicity values showing the spectrum of antiviral activity of the drugs and their safety margin *in vitro*. A549 cells were used to assess the antiviral activity of prototype strains of all members of CVB. The selectivity index (SI) for each drug has been calculated using the maximum ED_{50} values for CVB1-6 calculated in our study to give a conservative estimate of minimum SI values. The SI for Hizentra was not calculated as enough cytotoxicity to reach CC_{50} could not be generated using this drug.

Drug name	[1]CC_{50}	[2]ED_{50}						[3]SI
	(µM)	Unit	CVB1	CVB2	CVB3	CVB4	CVB5	CVB6	
Azithromycin	577	µM	NE	NE	32	NE	NE	NE	18
Enviroxime	58	µM	0.2	0.2	0.3	0.1	0.2	0.1	193
Favipiravir	1069	µM	363	98	141	NE	164	380	2.8
Fluoxetine	60	µM	8.1	5.2	5.7	6.2	3.6	7.1	7
Guanidine Hydrochloride	1785	µM	4.4	4.2	4.4	2.9	2.8	6.0	297
Hizentra	NC	mg/ml	0.2	0.04	0.02	NC	NC	1.4	NC
Itraconazole	2498	µM	5.9	4.0	2.4	6.9	7.2	8.0	312
Lovastatin	80	µM	NE	0.1	NE	NE	NE	NE	800
Norfluoxetine	90	µM	6.7	4.8	5.5	4.5	3.3	6.3	13
Pleconaril	3111	µM	0.1	0.5	NE	0.3	0.04	0.3	6222
Ribavirin	8	nM	620	230	320	300	160	410	13

NE = Not effective
NC = Not calculated
[1]Cytotoxic concentration 50%
[2]Median effective dose, values have been interpolated using Prism 5.02
[3]Minimum antiviral Selectivity index = CC_{50}/Maximum ED_{50} recorded in our study

Figure 1: Dose-response curve for antiviral activity and the cytotoxicity of the tested compounds.

Drug concentrations are presented as Log(10) of either micromolar or mg/ml of the compounds indicated on the X-axis. The Y-axis represents the cell viability (%) compared the control experiments without the virus but in the presence of the drugs. Log(agonist) vs. response statistics package, based on a nonlinear regression method in Graph Pad Prism program (version 5.02), was used to generate the graphs. The horizontal dotted lines show the ED_{50} values of each drug, where 50% of the cells were kept alive because of the antiviral activity of the drugs. The vertical dotted line in each graph corresponds to the published serum concentrations of the drugs, reached by therapeutic doses of each drug, with their originally designated therapeutic use. The serum concentration for Enviroxime was not available since it was used as topical drops. Instead, the dose of Enviroxime drops has been reported here. For Guanidine Hydrochloride, the serum concentration was not reported since it is not used in humans. In other mice experiments however, the serum values for this compound have been reported. Here, Guanidine Hydrochloride served as a positive control for antiviral effect.

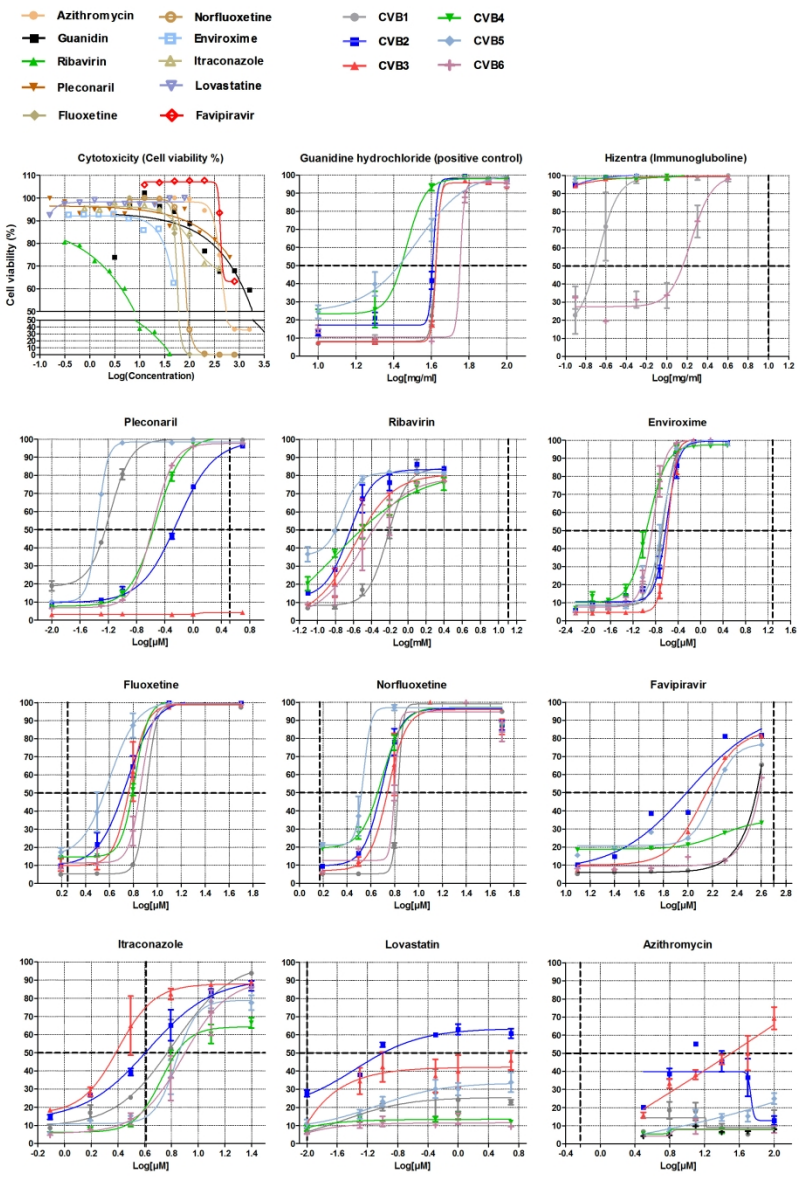
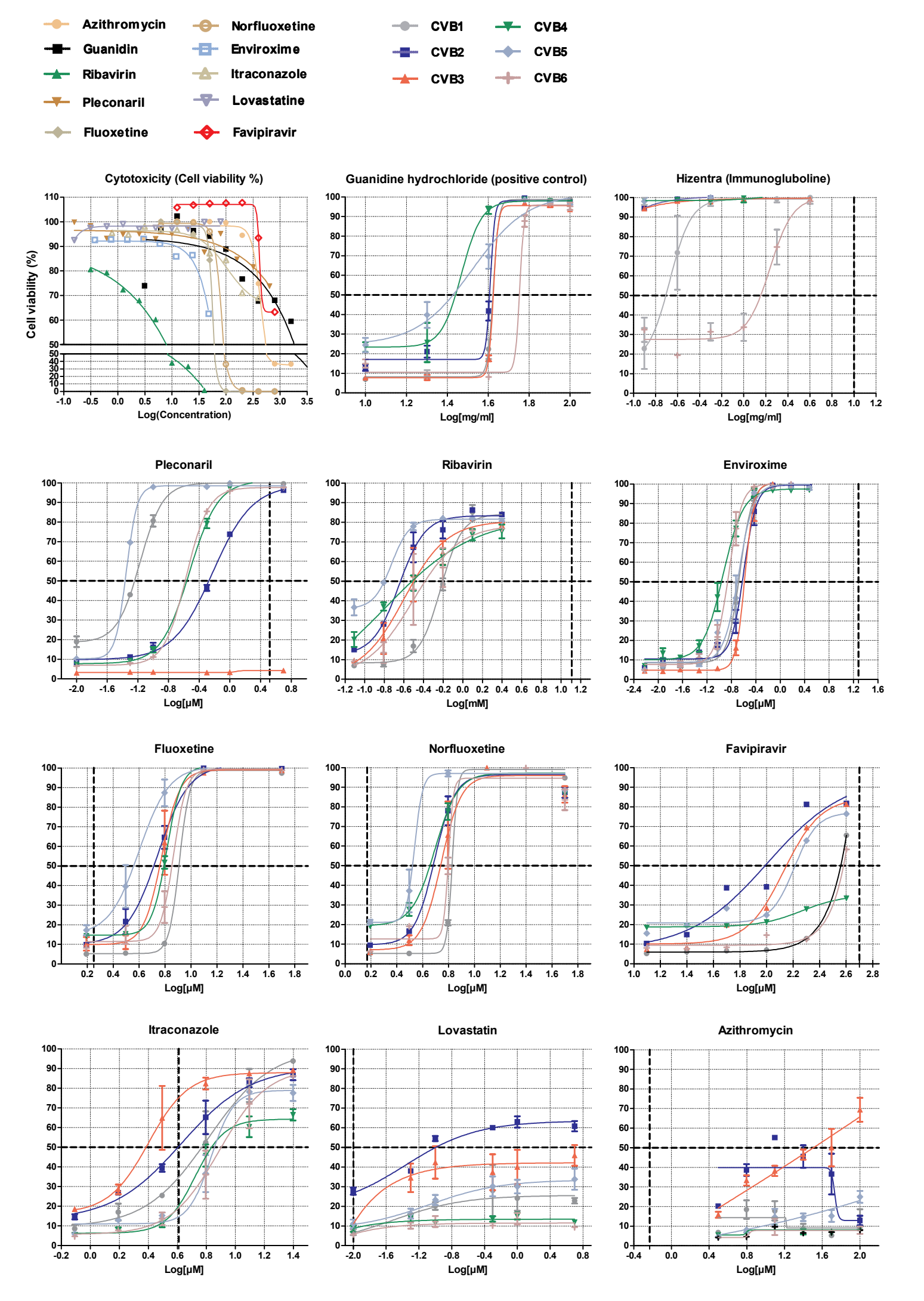


Figure 1: Dose-response curve for antiviral activity and the cytotoxicity of the tested compounds.Drug concentrations are presented as Log(10) of either micromolar or mg/ml of the compounds in the X axis. The Y axis represents the cell viability (%) compared the the control experiments without the virus but in the presence of the drugs. Log (agonist) vs. response statistics package based on nonlinear regression method in Graph Pad Prims program (version 5.02) was used to generate the graphs. The horizental dotted lines show the ED50 values of each drugs where the 50% of the cells kept alive because of the antiviral activity of the drugs. The vertical dotted line in each graph corresponds to the published serum concentrations of the drugs reached by theraputic doses of each drug for their origionally designated use. The serum concentration for Enviroxime was not available since it was used as topical drops and the dose of the drops has been reported here. For Guanidine the serum concentraion was not reported since it is not used in human, and here it served as a positive control for antiviral effect; hovever mice experiments reported the serum values for this compound.

205x288mm (300 x 300 DPI)


Azithromycin
Guanidin
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Pleconaril
Fluoxetine
Norfluoxetine
Enviroxime
Itraconazole
Lovastatine
Favipiravir
CVB1
CVB2
CVB3
CVB4
CVB5
CVB6
Cytotoxicity (Cell viability %)
Cell viability (%)
Log(Concentration)
Guanidine hydrochloride (positive control)
Log[mg/ml]
Hizentra (Immunogluboline)
Log[mg/ml]
Pleconaril
Log[µM]
Ribavirin
Log[mM]
Enviroxime
Log[µM]
Fluoxetine
Log[µM]
Norfluoxetine
Log[µM]
Favipiravir
Log[µM]
Itraconazole
Log[µM]
Lovastatin
Log[µM]
Azithromycin
Log[µM]

Supplementary Table 1: Summary of virus strains used in the study.

All viruses were ATCC prototype strains.

Virus type	Strain	Isolation history
CVB1	Conn-5	Stool from patient with aseptic meningitis, Connecticut, USA, 1948
CVB2	Ohio-1	Stool from patient with summer grippe, Ohio, USA, 1947
CVB3	Nancy	Stool from febrile patient with minor illness, Connecticut, USA, 1949
CVB4	J.V.B. (Benschoten)	Stool from 10-year-old child, New York, USA, 1951
CVB5	Faulkner	Child with suspected poliomyelitis, Kentucky, USA, 1952
CVB6	Schmitt	Rectal swab from 25-year-old healthy American male, Luzon, Philippine Islands, 1953

Supplementary Table 2: Summary of the drugs and their use in our study.

The drugs and the methods used to test their antiviral efficacy are presented here. The references cited here are the first reports of the antiviral property of the drugs and the reference for their serum concentrations when used for their clinical indications at the recommended dose. All the serum concentration values have been converted to molarity to be comparable with the reported results of the dose response curves and the ED_{50} and CC_{50} values.

Drug name	Provider	Product ID	Concentration (µM)	Reported serum Concentrations			Pre-incubation (hours)
				Concentration		[a]MW	
				As reported	µM		
Azithromycin (1)	Sigma	PZ0007	3.13-100	460 ng/ml (2)	0.6	785.02	24
Enviroxime (3)	ATI	4933161	0.05-1.5	[b]6.8 µg/ml (4)	19	358.41	1
Favipiravir (5,6)	CT	C8705	12.5-400	83 µg/ml (7)	528	157.1	1
Fluoxetine (8)	Sigma	F132	1.56-50	620 ng/ml (9)	1.8	345.79	1
Guanidine [C]HCl (10)	Sigma	G3272	1.04-10.4	-	-	95.53	0
Hizentra (11)	Behring	Pharmacy	0.12-4	710 mg/ml (12)	-	-	0
Itraconazole (13,14)	Sigma	I6657	0.78-25	2.85 µg/ml (15)	4.0	705.63	1
Lovastatin (16)	Sigma	1370600	0.01-5	4 ng/ml (17)	0.01	404.54	24
Norfluoxetine (8)	Sigma	F133	1.56-50	496 ng/ml (9)	1. 5	331.76	1
Pleconaril (18)	Sigma	SML0307	0.05-5	1.27 µg/ml (19)	3.3	381.35	0
Ribavirin (20)	Sigma	R9644	80-2500	3.2 µg/ml (21)	10	244.20	1

[a]Molecular weight of the drug
[b]Concentration used in the form of topical droplets, not serum concentration
[C]Hydrochloride
Sigma = Sigma-Aldrich
ATI = Advanced Technology and Industrial CO. LTD, Hong Kong
CT = Cellagen Technology, San Diego, USA
Behring = CSL Behring UK Limited

References

(1) Gielen V, Johnston SL, Edwards MR. Azithromycin induces anti-viral responses in bronchial epithelial cells. Eur Respir J 2010 Sep;36(3):646-654.

(2) Matzneller P, Krasniqi S, Kinzig M, Sorgel F, Huttner S, Lackner E, et al. Blood, tissue, and intracellular concentrations of azithromycin during and after end of therapy. Antimicrob Agents Chemother 2013 Apr;57(4):1736-1742.

(3) Heinz BA, Vance LM. The antiviral compound enviroxime targets the 3A coding region of rhinovirus and poliovirus. J Virol 1995 Jul;69(7):4189-4197.

(4) Phillpotts RJ, Wallace J, Tyrrell DA, Tagart VB. Therapeutic activity of enviroxime against rhinovirus infection in volunteers. Antimicrob Agents Chemother 1983 May;23(5):671-675.

(5) Furuta Y, Takahashi K, Fukuda Y, Kuno M, Kamiyama T, Kozaki K, et al. In vitro and in vivo activities of anti-influenza virus compound T-705. Antimicrob Agents Chemother 2002 Apr;46(4):977-981.

(6) Furuta Y, Komeno T, Nakamura T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. Proc Jpn Acad Ser B Phys Biol Sci 2017;93(7):449-463.

(7) Nguyen TH, Guedj J, Anglaret X, Laouenan C, Madelain V, Taburet AM, et al. Favipiravir pharmacokinetics in Ebola-Infected patients of the JIKI trial reveals concentrations lower than targeted. PLoS Negl Trop Dis 2017 Feb 23;11(2):e0005389.

(8) Ulferts R, van der Linden L, Thibaut HJ, Lanke KH, Leyssen P, Coutard B, et al. Selective serotonin reuptake inhibitor fluoxetine inhibits replication of human enteroviruses B and D by targeting viral protein 2C. Antimicrob Agents Chemother 2013 Apr;57(4):1952-1956.

(9) Pope S, Zaraa SG. Serum fluoxetine and norfluoxetine levels support the safety of fluoxetine in overdose. Ann Gen Psychiatry 2016 Nov 9;15:30-016-0117-z. eCollection 2016.

(10) Barton DJ, Flanegan JB. Synchronous replication of poliovirus RNA: initiation of negative-strand RNA synthesis requires the guanidine-inhibited activity of protein 2C. J Virol 1997 Nov;71(11):8482-8489.

(11) Biesert L. Virus validation studies of immunoglobulin preparations. Clin Exp Rheumatol 1996 MayJun;14 Suppl 15:S47-52.

(12) Niebur HB, Duff CM, Shear GF, Nguyen D, Alberdi TK, Dorsey MJ, et al. Efficacy and tolerability of 16% subcutaneous immunoglobulin compared with 20% subcutaneous immunoglobulin in primary antibody deficiency. Clin Exp Immunol 2015 Sep;181(3):441-450.

(13) Gao Q, Yuan S, Zhang C, Wang Y, Wang Y, He G, et al. Discovery of itraconazole with broad-spectrum in vitro antienterovirus activity that targets nonstructural protein 3A. Antimicrob Agents Chemother 2015 May;59(5):2654-2665.

(14) Strating JR, van der Linden L, Albulescu L, Bigay J, Arita M, Delang L, et al. Itraconazole inhibits enterovirus replication by targeting the oxysterol-binding protein. Cell Rep 2015 Feb 3;10(4):600-615.

(15) Abuhelwa AY, Foster DJ, Mudge S, Hayes D, Upton RN. Population pharmacokinetic modeling of itraconazole and hydroxyitraconazole for oral SUBA-itraconazole and sporanox capsule formulations in healthy subjects in fed and fasted states. Antimicrob Agents Chemother 2015 Sep;59(9):5681-5696.

(16) Werner B, Dittmann S, Funke C, Uberla K, Piper C, Niehaus K, et al. Effect of lovastatin on coxsackievirus B3 infection in human endothelial cells. Inflamm Res 2014 Apr;63(4):267-276.

(17) Kivisto KT, Kantola T, Neuvonen PJ. Different effects of itraconazole on the pharmacokinetics of fluvastatin and lovastatin. Br J Clin Pharmacol 1998 Jul;46(1):49-53.

(18) Pevear DC, Tull TM, Seipel ME, Groarke JM. Activity of pleconaril against enteroviruses. Antimicrob Agents Chemother 1999 Sep;43(9):2109-2115.

(19) Kearns GL, Abdel-Rahman SM, James LP, Blowey DL, Marshall JD, Wells TG, et al. Single-dose pharmacokinetics of a pleconaril (VP63843) oral solution in children and adolescents. Pediatric Pharmacology Research Unit Network. Antimicrob Agents Chemother 1999 Mar;43(3):634-638.

(20) Sadeghipour S, Bek EJ, McMinn PC. Ribavirin-resistant mutants of human enterovirus 71 express a high replication fidelity phenotype during growth in cell culture. J Virol 2013 Feb;87(3):1759-1769.

(21) Wu LS, Rower JE, Burton JR,Jr, Anderson PL, Hammond KP, Baouchi-Mokrane F, et al. Population pharmacokinetic modeling of plasma and intracellular ribavirin concentrations in patients with chronic hepatitis C virus infection. Antimicrob Agents Chemother 2015 Apr;59(4):2179-2188.