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Antifungal azoles itraconazole and posaconazole exhibit potent in vitro antiviral activity against clinical isolates of parechovirus A3 (*Picornaviridae*)☆☆

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Abstract

Parechovirus A3 (Par-A3, formerly human parechovirus 3) is an emerging viral infection of the central nervous system in children. We used an automated, homogeneous, cell based assay to identify itraconazole and posaconazole as inhibitors of Par-A3, with antiviral activity below concentrations clinically attainable in pediatric patients. Currently, there is no approved antiviral treatment for Par-A3 infection, despite numerous reports of serious Par-A3 disease in neonates and infants.

Parechovirus infections are common during the first years of life and the most commonly circulating parechovirus (Par-A1), mainly causes mild gastrointestinal and respiratory disease although more severe disease can be observed in young children (Harvala and Simmonds, 2009). Par-A3 is known to cause sepsis, meningitis, encephalitis, central nervous system infection, and sudden death in infants (Boivin et al., 2005; Felsenstein et al., 2014; Selvarangan et al., 2011; Shoji et al., 2013; Yuzurihara et al., 2013).

Par-A3 is readily detected in cerebrospinal fluid (CSF) (Harvala et al., 2011; Sharp et al., 2013; Walters et al., 2011) and is the most common parechovirus recovered from CSF (Harvala et al., 2009). Surveillance studies during 2000–2007 in Netherlands (van der Sanden et al., 2008), 2005–2010 in United Kingdom (Harvala et al., 2011), 2009–2012 in Denmark (Fischer et al., 2014), 2009–2013 in United States (Abedi et al., 2015), 2013–2014 in Australia (Cumming et al., 2015) and 2014 in Japan (Yamamoto et al., 2015), identified Par-A3 as the most prevalent parechovirus type in CNS-related infections in young children. This emphasizes the importance of Par-A3 as an agent of serious infections in young children; however, no specific therapies are available to treat Par-A3-infected children.

There is currently no approved antiviral drug available for diseases caused by enterovirus infection and treatment is limited to supportive care (van der Linden et al., 2015). Previous studies have shown that antifungal azoles itraconazole (ITZ) and posaconazole (PSZ) are

☆☆The use of trade names is for identification purposes only and does not constitute an endorsement by the Centers for Disease Control and Prevention or US Government.

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broad-spectrum inhibitors against a variety of enteroviruses (Gao et al., 2015; Strating et al., 2015). ITZ also inhibited a human rhinovirus in a murine model (Shim et al., 2016), which suggests that ITZ has potential as a treatment for picornavirus infection.

Par-A3 was detected and identified by RT-PCR, followed by Sanger sequencing of the amplicons from CSF specimens collected from infants ≤56 days old (median 22.5) seen in the 2012 and 2014 outbreak at Children's Mercy Hospital in Kansas City, Missouri. Par-A3 was isolated in Vero (African green monkey kidney, ATCC CRL-1586) cells from eleven of 32 Par-A3 positive CSF specimens which were subsequently used for drug susceptibility testing.

Compounds with antiviral activity against enteroviruses were tested for their ability to inhibit cytopathic effect (CPE) induced by Par-A1-A3 in an automated, homogeneous, cell-based assay that measured CPE in Vero cells as previously described (Rhoden et al., 2015) with minor modifications (Table 1).

Antifungal azole compounds itraconazole (ITZ) (I6657), posaconazole (PSZ) (32103), fluconazole (F8929), voriconazole (PZ0005) and ketoconazole (K1003), were purchased from Sigma-Aldrich (St. Louis, MO). The sources for additional compounds tested were as previously described (Rhoden et al., 2015). ITZ and PSZ exhibited antiviral activity against a reference Par-A3 strain (US-WI-09), with EC_{50} values of 1.09 ± 0.39 and 0.241 ± 0.024 μM, respectively (Table 1). Other tested antifungal azoles failed to exhibit antiviral activity against any of the three reference viruses from 10 to 70 μM, the highest concentration tested (Table 1).

The cellular receptors for Par-A are largely undetermined. Integrins are cell-surface receptors that mediate cellular adhesion to the extracellular matrix and cell-cell interactions that a wide variety of picornaviridae use as the cellular receptor. Many integrins recognize the RGD motif. $\alpha_v\beta_3$-integrin is the cellular receptor for Par-A1, but this has not been proven for Par-A2, -A4, -A5, or -A6 (Boonyakiat et al., 2001). This group of Par-A are relatively easy to grow in cell culture. Par-A3 and Par-A7 through -A17 all lack the RGD motif. Par-A3 can be isolated in cell culture (Vero cells), but Par-A7 through Par-A17 have not been grown in cell culture to date.

Antiviral activity of ITZ and PSZ against Par-A3 was confirmed in an automated, cell-based assay that used a checkerboard dilution matrix (cross-titration) of each compound and virus combination. ITZ, PSZ, enviroxime and 25-hydroxycholesterol (25HC) had broad-spectrum enterovirus (EV) species A-D antiviral activity, consistent with a similar mechanism of action against EV. Only ITZ and PSZ were identified as specific inhibitors of Par-A3 activity and had no detectable antiviral activity against other culturable parechoviruses (Par-A1 to A6), suggesting a different and yet unknown mechanism for Par-A3 antiviral activity (Table 2).

ITZ and PSZ exhibited potent antiviral activity against a reference strain of Par-A3 (US-WI-09) and eleven isolated 2012 and 2014 strains of Par-A3, but voriconazole, fluconazole, and ketoconazole failed to inhibit any of these strains (Table 3), suggesting a structure

activity relationship that extended chemical side chains may be important for antiviral activity (18).

Plasma concentrations of 468 ± 244 ng/ml and 1123 ± 811 ng/ml were attainable in pediatric patients for PSZ (Doring et al., 2017; Heinz et al., 2016), and 1015 ± 692 ng/ml for ITZ (Abdel-Rahman et al., 2007). In our studies, PSZ and ITZ had antiviral activity against the tested Par-A3 strains at concentrations clinically attainable in pediatric patients (Table 3).

Although our results were limited to in vitro studies, they indicate that FDA-approved antifungal drugs ITZ and PSZ have broad-spectrum EV and specific antiviral activity against Par-A3 only (Table 2). Our studies are the first to identify FDA-approved drugs that have Par-A3 antiviral activity. Clinical studies of ITZ (de Repentigny et al., 1998; Groll et al., 2002; Schmitt et al., 2001) and PSZ (Bernardo et al., 2013; Doring et al., 2012, 2017) have shown that these drugs are safe and well tolerated without severe side effects in pediatric patients. These results support the further evaluation of the use of ITZ and PSZ as antivirals against Par-A3.

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References

Abdel-Rahman SM, Jacobs RF, Massarella J, Kauffman RE, Bradley JS, Kimko HC, Kearns GL, Shalayda K, Curtin C, Maldonado SD, Blumer JL, 2007. Single-dose pharmacokinetics of intravenous itraconazole and hydroxypropyl-beta-cyclodextrin in infants, children, and adolescents. Antimicrob. Agents Chemother 51, 2668–2673. [PubMed: 17517842]

Abedi GR, Watson JT, Pham H, Nix WA, Oberste MS, Gerber SI, 2015. Enterovirus and human parechovirus surveillance - United States, 2009–2013. MMWR Morb. Mortal. Wkly. Rep 64, 940–943. [PubMed: 26334674]

Bernardo VA, Cross SJ, Crews KR, Flynn PM, Hoffman JM, Knapp KM, Pauley JL, Molinelli AR, Greene WL, 2013. Posaconazole therapeutic drug monitoring in pediatric patients and young adults with cancer. Ann. Pharmacother 47, 976–983. [PubMed: 23737511]

Boivin G, Abed Y, Boucher FD, 2005. Human parechovirus 3 and neonatal infections. Emerg. Infect. Dis 11, 103–105. [PubMed: 15705330]

Boonyakiat Y, Hughes PJ, Ghazi F, Stanway G, 2001. Arginine-glycine-aspartic acid motif is critical for human parechovirus 1 entry. J. Virol 75, 10000–10004. [PubMed: 11559835]

Cumming G, Khatami A, McMullan BJ, Musto J, Leung K, Nguyen O, Ferson MJ, Papadakis G, Sheppeard V, 2015. Parechovirus genotype 3 outbreak among infants, New South Wales, Australia, 2013–2014. Emerg. Infect. Dis 21, 1144–1152. [PubMed: 26082289]

de Repentigny L, Ratelle J, Leclerc JM, Cornu G, Sokal EM, Jacqmin P, De Beule K, 1998. Repeated-dose pharmacokinetics of an oral solution of itraconazole in infants and children. Antimicrob. Agents Chemother 42, 404–408. [PubMed: 9527794]

Doring M, Cabanillas Stanchi KM, Klinker H, Eikemeier M, Feucht J, Blaeschke F, Schwarze CP, Ebinger M, Feuchtinger T, Handgretinger R, Heinz WJ, 2017. Posaconazole plasma concentrations in pediatric patients receiving antifungal prophylaxis during neutropenia. Med. Mycol 55, 375–384. [PubMed: 27703016]

Doring M, Muller C, Johann PD, Erbacher A, Kimmig A, Schwarze CP, Lang P, Handgretinger R, Muller I, 2012. Analysis of posaconazole as oral antifungal prophylaxis in pediatric patients under 12 years of age following allogeneic stem cell transplantation. BMC Infect. Dis 12, 263. [PubMed: 23082876]

Felsenstein S, Yang S, Eubanks N, Sobrera E, Grimm JP, Aldrovandi G, 2014. Human parechovirus central nervous system infections in southern California children. Pediatr. Infect. Dis. J 33, e87–91. [PubMed: 24104958]

Fischer TK, Midgley S, Dalgaard C, Nielsen AY, 2014. Human parechovirus infection, Denmark. Emerg. Infect. Dis 20, 83–87. [PubMed: 24377661]

Gao Q, Yuan S, Zhang C, Wang Y, Wang Y, He G, Zhang S, Altmeyer R, Zou G, 2015. Discovery of itraconazole with broad-spectrum in vitro antienterovirus activity that targets nonstructural protein 3A. Antimicrob. Agents Chemother 59, 2654–2665. [PubMed: 25691649]

Groll AH, Wood L, Roden M, Mickiene D, Chiou CC, Townley E, Dad L, Piscitelli SC, Walsh TJ, 2002. Safety, pharmacokinetics, and pharmacodynamics of cyclodextrin itraconazole in pediatric patients with oropharyngeal candidiasis. Antimicrob. Agents Chemother 46, 2554–2563. [PubMed: 12121932]

Harvala H, McLeish N, Kondracka J, McIntyre CL, McWilliam Leitch EC, Templeton K, Simmonds P, 2011. Comparison of human parechovirus and enterovirus detection frequencies in cerebrospinal fluid samples collected over a 5-year period in edinburgh: HPeV type 3 identified as the most common picornavirus type. J. Med. Virol 83, 889–896. [PubMed: 21412796]

Harvala H, Robertson I, Chieochansin T, McWilliam Leitch EC, Templeton K, Simmonds P, 2009. Specific association of human parechovirus type 3 with sepsis and fever in young infants, as identified by direct typing of cerebrospinal fluid samples. J. Infect. Dis 199, 1753–1760. [PubMed: 19456229]

Harvala H, Simmonds P, 2009. Human parechoviruses: biology, epidemiology and clinical significance. J. Clin. Virol 45, 1–9. [PubMed: 19372062]

Heinz WJ, Cabanillas Stanchi KM, Klinker H, Blume O, Feucht J, Hartmann U, Feuchtinger T, Lang P, Handgretinger R, Doring M, 2016. Posaconazole plasma concentration in pediatric patients receiving antifungal prophylaxis after allogeneic hematopoietic stem cell transplantation. Med. Mycol 54, 128–137. [PubMed: 26483433]

Rhoden E, Zhang M, Nix WA, Oberste MS, 2015. In Vitro efficacy of antiviral compounds against enterovirus D68. Antimicrob. Agents Chemother 59, 7779–7781. [PubMed: 26149998]

Schmitt C, Perel Y, Harousseau JL, Lemerle S, Chwetzoff E, le Moing JP, Levron JC, 2001. Pharmacokinetics of itraconazole oral solution in neutropenic children during long-term prophylaxis. Antimicrob. Agents Chemother 45, 1561–1564. [PubMed: 11302829]

Selvarangan R, Nzabi M, Selvaraju SB, Ketter P, Carpenter C, Harrison CJ, 2011. Human parechovirus 3 causing sepsis-like illness in children from midwestern United States. Pediatr. Infect. Dis. J 30, 238–242. [PubMed: 20948454]

Sharp J, Harrison CJ, Puckett K, Selvaraju SB, Penaranda S, Nix WA, Oberste MS, Selvarangan R, 2013. Characteristics of young infants in whom human parechovirus, enterovirus or neither were detected in cerebrospinal fluid during sepsis evaluations. Pediatr. Infect. Dis. J 32, 213–216. [PubMed: 23042051]

Shim A, Song JH, Kwon BE, Lee JJ, Ahn JH, Kim YJ, Rhee KJ, Chang SY, Cha Y, Lee YS, Kweon MN, Park KS, Kim DE, Cho S, Cho HJ, Ko HJ, 2016. Therapeutic and prophylactic activity of itraconazole against human rhinovirus infection in a murine model. Sci. Rep 6, 23110. [PubMed: 26976677]

Shoji K, Komuro H, Miyata I, Miyairi I, Saitoh A, 2013. Dermatologic manifestations of human parechovirus type 3 infection in neonates and infants. Pediatr. Infect. Dis. J 32, 233–236. [PubMed: 23190775]

Strating JR, van der Linden L, Albulescu L, Bigay J, Arita M, Delang L, Leyssen P, van der Schaar HM, Lanke KH, Thibaut HJ, Ulferts R, Drin G, Schlinck N, Wubbolts RW, Sever N, Head SA, Liu JO, Beachy PA, De Matteis MA, Shair MD, Olkkonen VM, Neyts J, van Kuppeveld FJ, 2015. Itraconazole inhibits enterovirus replication by targeting the oxysterol-binding protein. Cell Rep 10, 600–615. [PubMed: 25640182]

van der Linden L, Wolthers KC, van Kuppeveld FJ, 2015. Replication and inhibitors of enteroviruses and parechoviruses. Viruses 7, 4529–4562. [PubMed: 26266417]

van der Sanden S, de Bruin E, Vennema H, Swanink C, Koopmans M, van der Avoort H, 2008. Prevalence of human parechovirus in The Netherlands in 2000 to 2007. J. Clin. Microbiol 46, 2884–2889. [PubMed: 18614653]

Walters B, Penaranda S, Nix WA, Oberste MS, Todd KM, Katz BZ, Zheng X, 2011. Detection of human parechovirus (HPeV)-3 in spinal fluid specimens from pediatric patients in the Chicago area. J. Clin. Virol 52, 187–191. [PubMed: 21813319]

Yamamoto SP, Kaida A, Naito T, Hosaka T, Miyazato Y, Sumimoto S, Kohdera U, Ono A, Kubo H, Iritani N, 2015. Human parechovirus infections and child myositis cases associated with genotype 3 in Osaka City, Japan, 2014. J. Med. Microbiol 64, 1415–1424. [PubMed: 26358716]

Yuzurihara SS, Ao K, Hara T, Tanaka F, Mori M, Kikuchi N, Kai S, Yokota S, 2013. Human parechovirus-3 infection in nine neonates and infants presenting symptoms of hemophagocytic lymphohistiocytosis. J. Infect. Chemother 19, 144–148. [PubMed: 22569793]

Table 1

Efficacy of drugs and antiviral compounds against reference Par-A strains.

	Mean EC_{50} ± SD (μM)		
	Par-A3 (US-WI-09)	**Par-A1 (Harris)**	**Par-A2 (Williamson)**
EV/RV Capsid inhibitors			
Pleconaril[a]	> 10	> 10	> 10
Pocapavir[a]	> 10	> 10	> 10
Vapendavir[a]	> 10	> 10	> 10
EV/RV Protease inhibitors			
Rupintrivir[a]	> 10	> 10	> 10
V-7404	> 10	> 10	> 10
Influenza inhibitors			
Amantidine[b]	> 10	> 10	> 10
Arbidol[a,c]	> 10	> 10	> 10
Favipiravir[a]	> 10	> 10	> 10
Oseltamivir [b]	> 10	> 10	> 10
Selective serotonin reuptake inhibitor			
Fluoxetine[b]	> 10	> 10	> 10
Bronchodilator			
Formoterol fumarate[b]	> 10	> 10	> 10
Antifungals			
Itraconazole[b]	1.09 ± 0.39	> 10	> 10
Posaconazole[b]	0.24 ± 0.02	> 10	> 10
Voriconazole[b]	> 70	> 70	> 70
Fluconazole[b]	> 70	> 70	> 70
Ketoconazole[b]	> 70	> 70	> 70
Anti-malarial			
Mefloquine[b]	> 10	> 10	> 10
Anti-protozoal			
Nitazoxanide[b]	> 10	> 10	> 10

[a] Completed a Phase II clinical trial but not yet FDA-approved.

[b] FDA-approved for an indication other than EV/RV infection.

[c] Licensed for human use in Russia and China.

Table 2

Antiviral activity of compounds against representative Enterovirus, Par-A reference and clinical isolates.

Virus (strain)	Species	Cell line	EC_{50} (μM ± SD)			
			Itraconazole	Posaconazole	Enviroxime	25HC
EV-A71 (HuN13–1)[a]	Enterovirus-A	RD [c]	3.02 ± 1.01	5.44 ± 0.82	0.47 ± 0.13	0.58 ± 0.19
EV-A71 (AH-FY-04)[a]	Enterovirus-A	RD	3.53 ± 1.38	0.49 ± 0.12	0.38 ± 0.13	0.42 ± 0.01
CVB5 (Faulkner)	Enterovirus-B	HeLa [d]	0.42 ± 0.19	1.82 ± 0.73	1.64 ± 0.46	> 10
E11 (Gregory)	Enterovirus-B	RD	0.34 ± 0.04	1.01 ± 0.16	0.27 ± 0.12	4.69 ± 2.53
Sabin 1	Enterovirus-C	HeLa	1.32 ± 0.41	> 10	0.28 ± 0.04	1.61 ± 0.56
CVA24v (KW83)	Enterovirus-C	HeLa	1.31 ± 0.39	0.79 ± 0.24	0.75 ± 0.14	1.83 ± 0.31
EV-D68 (14–18956)[b]	Enterovirus-D	RD	3.15 ± 1.51	3.65 ± 1.73	0.18 ± 0.03	0.16 ± 0.08
EV-D68 (14–18952)[b]	Enterovirus-D	RD	> 10	6.22 ± 1.91	1.45 ± 0.53	0.43 ± 0.02
Par-A1 (Harris)	Parechovirus-A	Vero [e]	> 10	> 10	> 10	> 10
Par-A2 (Williamson)	Parechovirus-A	Vero	> 10	> 10	> 10	> 10
Par-A3 (US-WI-09)	Parechovirus-A	Vero	1.17 ± 0.35	0.13 ± 0.01	> 10	> 10
Par-A4 (T75–4077)	Parechovirus-A	Vero	> 10	> 10	> 10	> 10
Par-A5 (T75–15)	Parechovirus-A	Vero	> 10	> 10	> 10	> 10
Par-A6 (US-WI-10)	Parechovirus-A	Vero	> 10	> 10	> 10	> 10

[a] Isolate from China.

[b] 2014 outbreak strain, US-IL.

[c] Human rhabdomyosarcoma cells.

[d] Human epithelial cervical carcinoma cells.

[e] African green monkey kidney cells.

Table 3

Antiviral activity of antifungal azoles against Par-A3 reference, 2012 and 2014 outbreak isolates.

Virus	Strain	Compound EC_{90} (μM ± SD), (ng/mL ± SD)				
		Itraconazole	**Posaconazole**	**Voriconazole**	**Fluconazole**	**Ketoconazole**
Par-A3	US-WI-09	1.17 ± 0.35 824 ± 247	0.135 ± 0.006 119 ± 44	> 10	> 10	> 10
Par-A3	12–06	1.02 ± 0.114 720 ± 80	0.112 ± 0.01 76 ± 4	> 10	> 10	> 10
Par-A3	12–07	1.55 ± 0.652 1094 ± 460	0.143 ± 0.013 100 ± 9	> 10	> 10	> 10
Par-A3	12–09	1.31 ± 0.017 924 ± 12	0.131 ± 0.002 91 ± 2	> 10	> 10	> 10
Par-A3	12–12	1.93 ± 0.682 1362 ± 481	0.139 ± 0.008 97 ± 6	> 10	> 10	> 10
Par-A3	12–13	0.433 ± 0.021 306 ± 15	0.129 ± 0.002 90 ± 1	> 10	> 10	> 10
Par-A3	12–15	1.08 ± 0.035 760 ± 25	0.135 ± 0.004 94 ± 1	> 10	> 10	> 10
Par-A3	14–01	0.522 ± 0.075 369 ± 67	0.141 ± 0.005 99 ± 4	> 10	> 10	> 10
Par-A3	14–03	0.798 ± 0.009 824 ± 247	0.122 ± 0.074 119 ± 44	> 10	> 10	> 10
Par-A3	14–05	0.408 ± 0.021 288 ± 15	0.137 ± 0.007 96 ± 5	> 10	> 10	> 10
Par-A3	14–08	0.487 ± 0.087 344 ± 61	0.421 ± 0.048 295 ± 33	> 10	> 10	> 10
Par-A3	14–14	0.138 ± 0.012 97 ± 9	0.136 ± 0.006 95 ± 5	> 10	> 10	> 10