Progress in the Development of Poliovirus Antiviral Agents and Their Essential Role in Reducing Risks That Threaten Eradication

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Chronic prolonged excretion of vaccine-derived polioviruses by immunodeficient persons (iVDPV) presents a personal risk of poliomyelitis to the patient as well as a programmatic risk of delayed global eradication. Poliovirus antiviral drugs offer the only mitigation of these risks. Antiviral agents may also have a potential role in the management of accidental exposures and in certain outbreak scenarios. Efforts to discover and develop poliovirus antiviral agents have been ongoing in earnest since the formation in 2007 of the Poliovirus Antivirals Initiative. The most advanced antiviral, pocapavir (V-073), is a capsid inhibitor that has recently demonstrated activity in an oral poliovirus vaccine human challenge model. Additional antiviral candidates with differing mechanisms of action continue to be profiled and evaluated preclinically with the goal of having 2 antivirals available for use in combination to treat iVDPV excreters.

Keywords. poliovirus; antiviral.

A Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO)–sponsored workshop held by a Committee of the National Research Council (NRC) in November 2005 concluded that “it would be prudent to develop at least 1, but preferably 2, polio antiviral drugs as a supplement to the tools currently available for the control of poliomyelitis outbreaks in the posteraicdiation era. Such antiviral drugs, in combination with stockpiled vaccines, would provide the ability to respond to a future accidental or intentional reintroduction of poliovirus . . ..” [1]. The immediate need identified by the Committee was for an antiviral drug or drugs to treat persons with B-cell immunodeficiencies who are excreting immunodeficiency-associated, vaccine-derived poliovirus (iVDPV). Persistent iVDPV excretion presents a personal risk of poliomyelitis to the patient as well as a programmatic risk of delayed global eradication. Since this topic’s last review [2], the CDC, WHO, Rotary International, the National Institutes of Health (NIH), the Food and Drug Administration (FDA), and the Bill & Melinda Gates Foundation, in conjunction with the Task Force for Global Health, have supported poliovirus antiviral development through the Poliovirus Antiviral Initiative (PAI). Here we update the considerable progress made in the discovery and development of poliovirus antiviral agents.

ESSENTIAL ROLE FOR POLIOVIRUS ANTIVIRALS IN ERADICATION EFFORT

Persons with primary B-cell immunodeficiency syndromes (PID) who received the live attenuated oral poliovirus vaccine (OPV) may spontaneously resolve their infection after a period of excretion that can vary from months to years, even decades [3–6], or continue to excrete vaccine-derived virulent virus into the environment until their death due to complications of...
immunodeficiency or poliomyelitis [7–10]. iVDPVs may be excreted at levels similar to those of acute poliovirus infections and exhibit the same reversion mutations away from attenuation seen in circulating vaccine-derived polioviruses (cVDPVs) although, unlike cVDPVs, iVDPV isolates rarely exhibit recombination with other species C enteroviruses [3].

Since the introduction of the OPV in 1961, approximately 65 individuals excreting iVDPV have been identified, principally through acute flaccid paralysis (AFP) surveillance and passive reporting to WHO [3]. The actual number of persons excreting iVDPV, however, is unknown. Halsey et al. [4] surveyed a convenience sample of 306 PID patients from immunodeficiency clinics in the United States, United Kingdom, Mexico, and Brazil and found poliovirus being excreted by 4 (1.3%) patients. In a subsequent study, no evidence of prolonged excretion was found in persons with residual paralytic poliomyelitis in Ethiopia, Pakistan, and Guatemala [5]. In 2009, WHO initiated pilot studies to estimate the prevalence of iVDPV excretion among persons with B-cell deficiency disorders in selected middle- and low-income countries. While this study is ongoing, results from the first 584 patients enrolled from 9 countries found 20 (3.4%) who were excreting poliovirus [11]. Tebbens and coworkers assumed a 1.0% infection rate in the PID population in “upper-income countries” and arrived at an estimate for the number of iVDPV long-term excreters worldwide to be 140 [12]. PAI has initiated a study in collaboration with The Jeffrey Modell Foundation, WHO, and CDC to determine the prevalence of poliovirus excretion in asymptomatic PID patients from 22 countries that currently use OPV. With the implementation of more active surveillance programs and identification of asymptomatic excreters, more accurate estimates of iVDPV patient prevalence will emerge, allowing for improved quantitative estimates of the programmatic risk to eradication posed by iVDPV excreters.

OTHER POSSIBLE ROLES FOR ANTIVIRALS

An antiviral drug could be employed for postexposure prophylaxis following an accidental poliovirus exposure in manufacturing or other posteradication essential poliovirus facilities. While such accidents are rare, published and anecdotal accounts indicate they have occurred in different sites around the world [13]. In some cases exposures were high with evidence of infection. The availability of an antiviral drug to potentially prevent exposed individuals from having to be placed into quarantine is considered an important use of an antiviral agent [2].

A third possible use of an antiviral could be to reduce transmission and prevent paralysis in the event of an outbreak while immunity develops to a simultaneously administered vaccine [1, 2]. Due to the potential for broad use across all age groups, antivirals used for outbreak control would pose a different risk and benefit analysis and likely require a larger safety database prior to approval for use in this scenario.

PROGRESS IN THE DEVELOPMENT OF POLIOVIRUS ANTIVIRALS

Polio eradication will not be considered complete until all poliovirus excretion is stopped. An effective poliovirus antiviral drug (or drugs) is the only option for iVDPV excreters. However, in the absence of a commercial market for a poliovirus antiviral product, there is little incentive for pharmaceutical companies to pursue drug development. The NRC recommended that a “public/private partnership” be formed “to provide the most efficient and least expensive means to develop antiviral drugs against polioviruses” [1]. This partnership was created in 2007 as the Poliovirus Antiviral Initiative (PAI). The mission of the PAI is to facilitate the rapid, cost-efficient development of at least 2 safe and effective poliovirus antiviral drugs that work by distinct mechanisms of action. Small molecules capable of inhibiting poliovirus replication in cell culture have been described since the early 1960s [14, 15] and comprise compounds of varying mechanisms of action, including those targeting virus-specific proteins, as well as compounds acting on host-cell proteins essential for viral replication. The CDC has evaluated many of these compounds. Most have been discarded due to insufficient potency or limited selectivity between cytoxicity and antiviral activity. Results to date have identified 2 compounds that meet the acceptance criteria for a poliovirus antiviral development candidate. One compound is the capsid inhibitor pocapavir (V-073), originally discovered at Schering-Plough (SCH 48973) [16], and licensed by ViroDefense Inc. The second compound is the 3C protease inhibitor AG7404 [17], an analog of rupintrivir [18], discovered by Agouron (Pfizer Inc), and now also being developed by ViroDefense Inc as V-7404. While pocapavir is presently being developed as a single-agent treatment, V-7404 is being positioned for combination treatment with pocapavir should drug resistance be an issue in treating PID patients with a single compound. The current status of these 2 compounds, as well as other earlier stage compounds, is summarized below.

POCAPAVIR

Pocapavir is a member of a class of compounds that inhibit replication of picornaviruses by preventing virus uncoating following virus attachment. The first of these “capsid inhibitors” that was shown to inhibit poliovirus replication in vitro was arildone [19, 20], which was shown to prevent paralysis and death when administered to poliovirus-infected mice [21]. Compounds with improved pharmaceutical properties had potent oral activity in the mouse model, even when dosing was initiated days after an intracerebral infection [22]. The hydrophobic binding site for capsid inhibitors (Figure 1) was first determined at atomic
resolution in a human rhinovirus [25] and later in poliovirus [23] in an early example of rational drug design.

Pocapavir was originally being developed by Schering-Plough to treat nonpolio enterovirus infections [16]. The program was terminated due to the insufficient commercial opportunity. ViroDefense and the CDC discovered the compound was very potent in vitro against polioviruses [26] (Figure 2). Pocapavir inhibited all 45 poliovirus strains tested, including wild, vaccine, vaccine-derived, and laboratory strains, in a cytopathic effect (CPE) assay. The concentration that reduced CPE by 50% (EC50) was included values ranging from 0.001 to 0.15 µM, with 90% of isolates inhibited at EC50 values of <0.056 µM [26].

All poliovirus isolates tested to date have been susceptible to pocapavir. Consequently, in order to understand the potential for drug resistance development, polioviruses were grown in laboratory cell cultures in the presence of pocapavir to allow for the selection of virus variants with reduced susceptibility to the drug [27]. The frequency of resistant variants in an otherwise susceptible virus population was determined independently for 5 poliovirus strains, and ranged from 1 in 2300 to 1 in 31 000. Sequence analysis of 160 independent resistant variants (80 isolates of poliovirus type 1, 40 isolates each of types 2 and 3) established that V-073 resistance involved a single amino acid change in either of 2 virus capsid proteins: VP1 (67 of 160 [42%]) or VP3 (93 of 160 [58%]). In resistant variants with a VP1 change, the majority (53 of 67 [79%]) exhibited a substitution of isoleucine at position 194 (equivalent position 192 in type 3) with either methionine or phenylalanine. Of those with a VP3 change, alanine at position 24 was replaced with valine exclusively (n = 93). The resistance phenotype was relatively stable upon passage of viruses in cell culture in the absence of drug. Single-step growth studies showed no substantial differences between drug-resistant variants and the virus stocks from which they were derived, while the resistant viruses were generally more thermally labile than the corresponding drug-susceptible parental viruses [27].

When assessed in mice, the drug-resistant variants were effectively neutralized by human OPV- and inactivated polio virus
vaccine (IPV)—induced immune serum samples [28]. Further, the drug-resistant variants elicited virus neutralizing antibody titers in CD-1 mice comparable to drug-susceptible parental and Sabin vaccine strain viruses. Infection efficiency of TgPVR21 mice by variants was, however, considerably lower (5 of 6 variants) than or comparable (1 of 6 variants) to that of the parental viruses. Drug-resistant variants replicated in the mice to levels substantially less than (5 of 6 variants) their drug-susceptible parental viruses and were on average 1.4 log\textsubscript{10} (range, 0.3 to >2.8 log\textsubscript{10}) less neurovirulent [28]. Polioviruses possessing either of the 2 amino acid substitutions that confer resistance to pocapavir have not been found in nature [26]. Moreover, a search of the CDC poliovirus sequence database failed to identify pocapavir resistance markers among 4500 VP1 and 250 VP3 sequences [CDC, unpublished data]. These observations suggest that in nature, there is a fitness cost associated with the pocapavir-resistant phenotype.

The virus-specific nature of the drug interaction with the capsid explains the lack of toxicity of capsid inhibitors in clinical studies in the treatment of picornavirus infections, including the rhinovirus common cold [29] and other enterovirus infections [30]. Human phase 1 single and 14-day multiple ascending dose studies demonstrated that pocapavir was well tolerated at total doses up to 1600 mg per day, the highest dose tested [Hincks and Collett, in preparation]. Pocapavir exposures (Maximal plasma concentration [C\textsubscript{max}] and area under the curve [AUC]) were increased 3–5 fold when administered with a high-fat meal, and the AUC was increased with twice daily dosing. Following a single 1600 mg dose with a high-fat meal, plasma levels remained well above the minimum inhibitory concentration required to inhibit the growth of 90% of poliovirus isolates (MIC\textsubscript{90}) throughout the dosing interval.

Pocapavir was evaluated for antiviral activity in a first-of-a-kind, randomized, double-blinded, placebo-controlled human monovalent oral poliovirus vaccine type 1 (mOPV1) challenge model. Preliminary results of this study were recently presented at the 2013 Interscience Conference on Antimicrobial Agents and Chemotherapy annual meeting [31] and will be described in detail elsewhere [Collett et al., manuscript in preparation]. Pocapavir recipients (day 45), all subjects had cleared virus. In summary, potent and rapid pocapavir antiviral activity was demonstrated in this human mOPV1 challenge model.

**V-7404**

The 3C protease inhibitor V-7404, which was first developed by Pfizer [17] as an antirhinovirus agent, also has potent activity against polioviruses in vitro [32] (Table 1). A phase 1 dose ranging study demonstrated V-7404 safety at single doses up to 2000 mg, but suggested limited oral bioavailability [17].

**COMBINATION ANTIVIRAL TREATMENT**

Treatment-emergent resistance is to be expected with any antiviral therapy. In the case of immunodeficient individuals, development of resistance may result in the inability to clear poliovirus. Capsid inhibitors have been used as monotherapy in the treatment of immunodeficient patients suffering from non-polio enterovirus infections without treatment failures due to resistance development [30]. In large studies of natural rhinovirus infections treated with other capsid inhibitors, resistance has been reported to be in the 3% range [33].

**Table 1. In vitro Profile of Lead Poliovirus Antiviral Agents**

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Pocapavir</th>
<th>V-7404</th>
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<tbody>
<tr>
<td>Mechanism</td>
<td>Capsid inhibitor</td>
<td>3C Protease inhibitor</td>
</tr>
<tr>
<td>In vitro cytotoxicity CC\textsubscript{50}</td>
<td>6 \textmu M</td>
<td>572 \textmu M</td>
</tr>
<tr>
<td>In vitro effectiveness EC\textsubscript{50} range</td>
<td>0.001 to 0.15 \textmu M (n = 45)</td>
<td>0.13 to 0.55 \textmu M (n = 45)</td>
</tr>
<tr>
<td>In vitro MIC\textsubscript{90}</td>
<td>0.056 \textmu M (32 ng/mL)</td>
<td>0.45 \textmu M (240 ng/mL)</td>
</tr>
<tr>
<td>In vitro resistance frequency</td>
<td>\texttimes 10\textsuperscript{-4} to \texttimes 10\textsuperscript{-5}</td>
<td>Approximately \texttimes 10\textsuperscript{-4}</td>
</tr>
<tr>
<td>Basis for in vitro resistance</td>
<td>Single amino acid change in capsid proteins VP1 or VP3</td>
<td>Single amino acid change in 3C protease</td>
</tr>
<tr>
<td>Combined in vitro resistance potency</td>
<td>Approximately \texttimes 10\textsuperscript{-8}</td>
<td>Synergistic</td>
</tr>
</tbody>
</table>

Abbreviations: CC\textsubscript{50}, concentration that is cytotoxic to 50% of host cells; EC\textsubscript{50}, half maximal effective concentration; MIC\textsubscript{90}, minimum inhibitory concentration required to inhibit the growth of 90% of poliovirus isolates.
Pocapavir and V-7404 have similar single agent in vitro resistance frequencies (approximately \(10^{-4}\) for both compounds). The in vitro resistance frequency of the combination was below the limit of detection \((<10^{-8})\), as would be expected of agents with a distinct mechanism of action [32]. A further benefit of the combination of pocapavir and V-7404 relates to the strong synergistic activity against the 3 Sabin strains observed in vitro. This effect is measurable at V-7404 concentrations as low as 20 nM for poliovirus 1 and 3, a concentration 20 times lower than is required when V-7404 is used alone (446 nM) [32]. This combination produces synergistic antipoliovirus effects and should greatly reduce or eliminate the potential for drug resistance for polioviruses that are typically found in peak concentrations of \(10^4\) to \(10^5\) plaque forming units per gram in human feces.

Both pocapavir and V-7404 demonstrated excellent safety profiles in nonclinical toxicology and phase 1 clinical studies. Based on the single-agent safety profile of each compound, it is expected that the combination of the 2 drugs will be well tolerated. The V-7404 plasma levels achieved in humans were slightly higher than MIC\(_{90}\) for V-7404 alone and well above the V-7404 concentration required to produce synergy when used in combination with V-073 [17, 32].

**COMBINATION ANTIVIRAL AND VACCINE TREATMENT**

After global withdrawal of all OPV, an antiviral drug might be coadministered with IPV in the event of a new outbreak of disease due to either wild viruses or VDPVs. In such a situation, the antiviral drug would provide immediate protection while the vaccine is eliciting an immune response. Immunization of mice with IPV with concurrent dosing of poliovirus antiviral V-073 showed no detrimental impact on the elicitation of serum neutralizing antibodies [34].

**MONOCLONAL ANTIBODIES**

A cocktail of 2 or 3 highly potent monoclonal antibodies with activity across all 3 serotypes may be a useful product to use in combination with pocapavir. The specific activity of the lead antipoliovirus antibody A12 is about 200,000 neutralizing units against polio 1 and 2 per milligram [35]. Circulating titers 100-fold higher than the levels induced by vaccine or more may be required to clear poliovirus. The site where poliovirus resides in chronically infected individuals is unknown and it is unclear how accessible to antibodies the virus may be. Therefore, for treatment of iVDPV excretors, it may be desirable to bring the level of neutralizing antibodies in blood to the highest levels, dictated by the maximum tolerated dose that will be determined in a phase 1 study. Work is planned to evaluate the effect of IV monoclonal antibody treatment on virus excretion in a primate model. If A12 monoclonal antibody is successful in increasing the rate of excretion cessation in the animal model, additional work will be done to build and characterize the ideal antibody combination which will provide non-cross-resistant neutralization of all relevant wild-type and vaccine PV strains with a minimum number of antibodies. The successful combination would be advanced into clinical studies, including assessing activity in the mOPV1 challenge model used with pocapavir.

**OTHER ANTIVIRAL CLASSES**

CDC continues to review the literature and scientific meetings and invites newly described anti-picornavirus compounds for profiling in their antiviral assays. Compounds showing activity at <1 \(\mu\)M against the 3 Sabin strains are profiled against a panel of 45 poliovirus isolates. Those showing an acceptable spectrum and potency are further characterized for resistance and activity against pocapavir-resistant virus.

**NEXT STEPS**

The progress made in the PAI program to date make possible the continued advancement of pocapavir as a single agent and the development of pocapavir combinations with V-7404 or monoclonal antibodies. The ability of pocapavir alone to clear infections in adult and pediatric iVDPV excreters will soon be assessed in a small pilot study. If acceptable activity is demonstrated, the study will be expanded to include a larger group of adult and pediatric patients as part of a study to support product registration. If virus clearance is not achieved and treatment-emergent resistance develops in the pilot study, further treatment of PID patients will await the availability of a second antiviral or monoclonal antibody to use in combination with pocapavir. In either case, the ability to effectively treat iVDPV excretors with antiviral agents is much closer to being realized.

**CONCLUSIONS**

Vaccine-derived polioviruses persistently excreted by iVDPV represent a risk for the reemergence of poliomyelitis in the post-eradication era. Poliovirus-specific antiviral agents represent the only option for reducing the personal risk of fatal iVDPV disease and the programmatic risk of a continuing source of virus transmission. Antiviral agents may also play an important role in postexposure prophylaxis following an accidental exposure and in certain outbreak scenarios. The PAI program has successfully identified and is developing potent and selective antiviral agents. The lead antiviral pocapavir recently demonstrated effectiveness in reducing the duration and level of poliovirus excretion in a mOPV1 challenge model. Poliovirus antivirals with differing mechanisms of action and monoclonal antibodies...
continue to be evaluated preclinically with the goal being the availability of 2 antiviral agents to be used in combination to treat iVDPV excretors should resistance development become an issue with monotherapy. With the availability of an efficacious treatment, iVDPV-excreting individuals should be more readily identified. Their effective treatment will serve to eliminate the risk to the patient of paralytic disease and remove a potential source of poliovirus, bringing us closer to a polio-free world.

Notes

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References


