ELSEVIER

Journal of Clinical Virology 39 (2007) 304–307

JOURNAL OF CLINICAL VIROLOGY

www.elsevier.com/locate/jcv

Short communication

Characterization of mutations in the VP_1 region of Sabin strain type 1 polioviruses isolated from vaccine-associated paralytic poliomyelitis cases in Iran

Pooneh Rahimi [a,*], H. Tabatabaie [a], Mohammad M. Gouya [b], Mohsen Zahraie [b], M. Mahmudi [a], A. Ziaie [c], K. Samimi Rad [a], Sh. Shahmahmudi [a], T. Musavi [b], T. Mokhtari Azad [a], R. Nategh [a]

[a] *Polio National Laboratory, Division of Virology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Iran*

[b] *The Disease Management Center of the Ministry of Health, Iran*

[c] *Institute of Biochemistry and Biophysics, University of Tehran, Iran*

Received 24 July 2006; received in revised form 15 April 2007; accepted 19 April 2007

Abstract

Background: The live-attenuated oral polio vaccine used to interrupt poliovirus transmission is genetically unstable. Reversion of some attenuating mutations, which normally occurs during vaccine strain replication in some recipients, and can rarely cause vaccine-associated paralytic poliomyelitis (VAPP). The poliovirus eradication program designed by the World Health Organization (WHO) includes immunization with OPV in addition to careful surveillance of all acute-flaccid paralysis (AFP) cases.

Objectives: In Iran we last isolated imported wild poliovirus in 2000 and the immunization coverage was 100% in 2002. During 2001, there were three AFP cases with residual paralysis from which Sabin-like type 1 polioviruses were isolated in our national polio laboratory.

Study design: The complete VP_1 region of the three isolates was sequenced and amino acid substitutions associated with these neurovirulent isolates were recorded.

Results: These isolates had either 4, 2 or 1 nucleotide substitution(s) in the VP_1 region, corresponding to amino acid change in the VP_1 of isolate 1 of either ($H \xrightarrow{149} Y$), ($T \xrightarrow{106} A$) or ($I \xrightarrow{90} L$), respectively.

Conclusions: Surveillance of the VAPP cases in countries where endemic transmission has recently ceased increases our understanding of the important neurovirulent mutations in vaccine-strain isolates and assists in planning the next step in the eradication program in these countries.

Keywords: Vaccine-like poliovirus; Vaccine-associated paralytic poliomyelitis; Acute-flaccid paralysis

1. Introduction

The three serotypes of poliovirus can all multiply in motor neurons and cause paralysis. Both inactivated and oral attenuated (OPV) vaccines are used to prevent poliomyelitis. Since 1961, routine and mass administration of OPV has prevented millions of cases of paralytic poliomyelitis (Kew et al., 2005). Although OPV is remarkably free of adverse effects in immunocompetent children, reversion of the attenuating base substitutions in OPV commonly occurs during virus replication in the human intestine. In rare instances these revertants cause vaccine-associated paralytic poliomyelitis (VAPP) (Kew and Nottay, 1984; Wimmer et al., 1993).

An effective strategy designed by the World Heath Organization to abolish the circulation of wild polioviruses has two major parts: mass immunization with OPV coupled with aggressive surveillance of acute flaccid paralysis (AFP) cases to determine the wild or vaccine-derived origin of isolated polioviruses (Dowdle et al., 2003).

National immunization days (NIDs) and sub-national immunization days (SNIDs) in addition to routine OPV

* Corresponding author. Tel.: +98 21 88950595; fax: +98 21 88950595.
E-mail address: prahimi@razi.tums.ac.ir (P. Rahimi).

1386-6532/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2007.04.017

Table 1
Specification of three acute-flaccid paralysis cases during the year 2001 and the results of intratypic differentiation tests

Lab no.	Age (m)	Sex	Province	OPV history	VAPP case classification[a]	Last OPV vaccination date	Onset date	Specimen date	ELISA[b]	RT-PCR[c]
1	48	F	Khorasan	3	Community[d]	14 October 1998	1 July 2001	26 July 2001	PV1-SL	PV1-S
3	40	M	Kerman	3	Recipient	12 June 2001	20 June 2001	25 June 2001	PV1-SL	PV1-S
5	24	M	Mazandaran	3	Recipient	5 July 2001	21 July 2001	29 July 2001	PV1-SL	PV1-S

[a] Patients with no known contact with each other or a mutual contact.
[b] SL: Sabin-like poliovirus, the results of ELISA test according to the WHO protocol.
[c] PV-S: Poliovirus-Sabin strain, the results of RT-PCR test according to the WHO protocol.
[d] Community case: a category used for patients neither recently vaccinated nor known to live in contact with individuals having received OPV.

immunization and AFP surveillance were established in Iran in 1994. The last case of indigenous wild poliovirus was identified in 1997; and in 2000, the last imported wild poliovirus was isolated. OPV coverage was 100% in 2002 (MMWR: Dec 14, 2001; Who/Unicef: Aug 2006).

In 2001, among all AFP patients there were three cases with residual paralysis from which Sabin-like type 1 polioviruses were isolated. We describe the genotypic analysis of these isolates and the mutations associated with their neurovirulence.

2. Materials and methods

2.1. Patients

Three patients with AFP had anti-poliovirus antibody determined after the Sabin-like type 1 polioviruses isolated from them during their convalescence.

2.2. Serology

Serum levels of anti-poliovirus antibody were determined by microneutralization (Table 1).

2.3. Specimens

Two stool samples taken 48 h apart were obtained from each patient within 14 days after the onset of AFP and stored at −20 °C.

2.4. Virus isolation and serotyping

Stored fecal specimens were processed and poliovirus identified on three cell lines (RD, $L_{20}B$, Hep_2). Cytopathic effect was detected after two passages at 14 days after inoculation. Viruses were typed by microneutralization using pools of antisera against polioviruses and other enteroviruses as recommended by WHO (WHO laboratory manual, 2004).

2.5. Intratypic differentiation tests

Intratypic differentiation of the poliovirus isolates was accomplished with an enzyme-linked immunosorbent assay (ELISA) using cross-absorbed intratypic-specific rabbit antisera that differentiated between non-Sabin and Sabin-derived strains. Serotypes were identified using RT-PCR and riboprobes which hybridized specifically with the genome of vaccine-related isolates. Isolates were sent to the Centers for Disease Control and Prevention (CDC) for sequencing.

2.6. Virus

The accession number of the reference Sabin-strain type 1 used for sequence analysis is AY082688.

2.7. Sequence analysis

Nucleotide sequence data from the isolates was analyzed using the Clustal W program.

3. Results

Each patient with AFP had received three doses of OPV. They lived in a different province and had no known contact or a mutual contact with other patients. Each had a titer of antibody against poliovirus type 1 of >120. Patient 1 was classified as a community-acquired case, since there was no recent vaccination or discernable contact with individuals having received OPV.

All three isolates were identified as Sabin strain type 1 poliovirus (Table 1).

Isolates 1, 3 and 5 had 99.5%, 99.8% and 99.9% identity, respectively with the VP_1 region of the reference Sabin strain. Isolate 1 had four nucleotide substitutions in the VP_1 region (A2659T, C2707T, C2924T and A3253G). Isolate 3 had two nucleotide substitutions in this region (A2795G and A3052G), and isolate 5 had one nucleotide substitution (A2747T). Table 2 summarizes the sequence analysis of these isolates. A comparison between the amino acid residues of the VP_1 protein of the isolates and the reference strain indicated that isolate 1 had one amino acid substitution [149(H → Y)]. Isolate 3 had one amino acid substitution [106(T → A)], and isolate 5 had one substitution [90(I → L)]. Table 3 shows the amino acid substitutions in the VP_1 protein in each isolate. The 5′-NCR of each isolate was sequenced and in all of the three isolates a (G → A) substitution in nucleotide 480 was found.