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Taiwan's First Vaccine-Derived Poliovirus Induced Paralysis Case

Abstract

On April 16, 2001, the Research and Laboratory Testing Division of the Center for Disease Control received a report of an acute flaccid paralysis (AFP) and serious enterovirus infection case. For paralysis, the case was referred to the Linkou Chang Gung Hospital. The case was placed under respirator for life maintenance. Initially, throat swab was collected from the case. The virus isolated was poliovirus 1. By regulations of the World Health Organization and the AFP monitoring system of the Center, two fecal specimens at 24-hour interval should be collected from the case. By virus isolation and neutralization testing of the fecal specimens, and gene sequencing comparison of virus at the VP1 region, it was found that the gene sequence of the isolated virus and the gene sequence of poliovirus 1 had a 3% difference at the VP1 region, it was then decided that the virus was vaccine-derived poliovirus (VDPV).

Introduction

Upon attack of poliovirus or other enteroviruses, the immune system of the human body will produce neutralization antibodies[1,2]. After infection (feces to

mouth transmission), poliovirus stays in intestinal tract for 4-8 weeks[3], and then the virus is excreted with feces. Chances of oral poliovirus vaccine (OPV) inducing vaccine-associated paralytic poliomyelitis (VAPP) in healthy persons are low, the vaccine is considered very safe. However, in immunodeficient persons, chances of getting VAPP are 3,000 times higher than healthy persons. Vaccine strains stay in these persons longer, and some cases may even result in death[4]. Recent studies have shown that asymptomatic immunodeficient patients or immunodeficient persons with vaccine-associated poliomyelitis symptoms may continue to discharge viruses for as long as ten years. Persons of immunodeficiency should, therefore, avoid taking OPV or exposing to vaccinated individuals. These children, however, are detected at later age; they are likely to have been vaccinated already.

In April 2001, the AFP monitoring system detected an eight-year boy with VAPP. Fecal specimens were collected at different times for testing. After the standard operational procedures for the handling of fecal specimens, the specimens were inoculated on RD, L20B and Hep-2 cells for culturing. After CPE, the virus was collected and neutralized with poliovirus antibody. Poliovirus 1 strain was isolated. Augmented by reverse transcriptase polymerase chain reaction (RT-PCR) for variability at the 5' non-cording and VP1 regions[5-7] to make sure there were PCR products, the products were then analyzed and compared for their components by gene sequencing method.

Materials and Methods

Immunodeficient Patient

This case was born in 1993, and was vaccinated OPV at 2, 4, 6, and 15 months and 6 years of age. Nothing abnormal was noticed. On April 6, 2001 (the case was in the second grade of primary school), the case was taken

to a clinic for fever, coughing and running nose. The case developed paralysis of the left hand, and flaccid right upper limb and both sides of the lower limbs on April 9; and on April 12, for unable to walk, was admitted to a hospital in Ilan (April 10-13), and then referred to the Linkou Chang Gung Hospital on April 13 for difficult swallowing. The AFP Investigation Committee reported on April 25 the following symptoms: paralysis, short breath, fever at 38℃, flaccid limbs, drooping eyelids, coughing, difficult swallowing, flaccid tongue, no reflex at biceps, triceps and knees. CFS testing showed WBC of 67, mainly lymphocytes; glucose was normal, protein at 125.7; and bacterial and virus culturing of cerebrospinal fluid and blood was negative. Initially, doctor suspected of serious enterovirus infection, and immediately administered intravenous immunoglobulin injection. Paralysis was not improved and unbalanced. Poliovirus 1 strain was isolated in the specimens. In May, the titer of the immune antibody was tested: IgG in serum was 270 mg/dl (normal value, 608-720 mg/dl), IgA<5.88 mg/dl (normal value, 33-236 mg/dl), IgM was 7.78 mg/dl (normal value, 43-207 mg/dl), and CD4/CD8 was 0.3 (normal value, 1.42-1.90). Tested six months later, the IgG was 270 mg/dl. The clinical doctor diagnosed the case as patient of immunodeficiency.

Isolation of virus and Type Assessment

Throat swabs on the fifth day after onset, and fecal specimens on the 17^{th,} 52nd, 54th, 179th, 261st and 337th days were collected for testing (Table 1). Specimens were handled by standard operational procedures[11], and then inoculated on RD, L20B and Hep-2 cells for virus isolation. After CPE, nine virus strains were isolated. They were then typed by poliovirus anti-serum neutralization test.

Gene Sequencing of Poliovirus

1.Extraction of RNA

RNA was extracted from the poliovirus 1 thus typed by the QIAmp viral RNA kit (Qiagen). 140 µl of virus solution was added in 560 µl buffer AVL, mixed evenly, left under room temperature for 10 minutes, and added 560 µl of absolute alcohol. The mixed fluid was passed twice through QIAmp spin columns. The column was washed with buffer AW1 and AW2, and finally resolved RNA with 60 µl of pure water.

2.RT-PCR

The RT-PCR method was used[8]. 5 µl of virus RCA was added to the PCR reaction tube, added 20 µM of positive and negative primers (Table 2), 0.2 mM of d-NTP, 2 mM of $MgCl_2$, Tris-HCl, 10 U of nucleic acid inhibition enzyme, 5 U of polymerase, , and added distilled water to a total volume of 50 µl for reaction at 42℃ for 30 minutes. cDNA was synthesized, and PCR was continued as follows: at 95℃ for 3 minutes, 35 reactions were conducted at 94℃ for 30 seconds, 50℃ for 30 seconds, and 70℃ for one minute, and finally at 70℃ for 10 minutes.

3. Sequencing Analysis

ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem) was used. A certain amount of nucleic acid , 3 µl of premix (including Tris-HCl buffer, pH 9.0, $MgCl_2$, dNTP mix, labeled A dye terminator, C dye terminator, G dye terminator, T dye terminator, Ampli Tag DNA polymerase FS with thermally stable pyrophosphatase), 3.2 pmole nucleic acid primer (Table 1), and added water to a total volume of 10 µl. The product was removed to a reactor preheated at 96℃ for one minute, and conducted 25 cycles of reaction at 96℃ for 10 seconds, 50℃ for 5 seconds, 60℃ for 4 minutes, and finally stopped at 4℃.

4. Purification of Reaction Product by Alcohol Sedimentation

Each centrifugal tube was added 10 μl of distilled water, 60 μl of pure alcohol and 5 μl of 125 mM EDTA, evenly mixed and left under room temperature (light-free) for 15 minutes, centrifuged at 15,000 g for 20 minutes, and sucked with tip the upper clear fluid. Each tube was then added 70 μl of 70% alcohol, left in refrigerator at 4℃ for 5 minutes, centrifuged at 15,000 g for 5 minutes. The product was dried with a vacuum dryer and dissolved in 10 μl of Hi-Di formamide.

Epidemiological Investigation and Monitoring

The case was then a second grader of a primary school. He is one of the four children in the family. The two elder sisters are in primary school 5th and 6th grades. They had OPV immunization when they were in the first grade. The younger brother is in kindergarten. He had his OPV following normal schedule. No paralysis of limbs was noted in children in the neighborhood and the siblings. Investigation by the Ilan County Health Bureau showed that, the classroom of the case, surnamed Lin, is on the third floor. No toilet was available then for the reconstruction of classrooms on the third floor, students had to share toilet on the second floor. In March 2001, six children of the first and the second grades had to have their make-up vaccination of OPV; three children on March 6, two on March 8, and one, for chickenpox, was not vaccinated. In March, a total of five children in the first and the second grades were given OPV make-up vaccination. The two in the second grade (classes 5 and 6 of the second grade) were not in the same class of the case (class 3 of the second grade), though they played together. The case stayed behind in the after-school class.

For investigation, the Health Bureau collected fecal specimens from the three sisters and one brother of the case. Laboratory testing showed all negative of

poliovirus, though adenovirus was isolated in the eldest sister's specimen. To be careful, more specimens were collected for testing, 33 children from class 3 of the second grade, 11 children from the Chunghua after-school class, and 15 from the community (neighbors), totaling 62. All specimens tested poliovirus negative (in two of the 33 children of class 3, adenovirus was isolated) (Table 3). Family members of the case, community and school were epeidemiologically investigated, including 15 family members, 14 in the community, 1,637 in the school (number of students, 1,554), and 16 of the after-school class (number of students, 11). No suspected cases were detected.

Control

The house of the case, the community, the school and the after-school class were disinfected. All children in the neighborhood had OPV. Follow-up of children under five years in the neighboring Li had been completed.

Measures recommended by the Advisory Committee on Immunization of the Department of Health

1. In the first six months after the onset of AFP, the case should be followed-up once every month; then once every two months after six months; and more long-term follow-ups thereafter.
2. Ilan County Health Bureau should try to improve the OPV vaccination coverage rate. If the coverage rate is lower than 90%, the use of mopping-up approach to improve coverage rate should be considered. Currently, the County has a coverage rate of more than 90%.

Follow-up of case after 60 days

1. Motor nerves: the amplitude of the median of both the left and the right sides had either greatly declined or disappeared; the amplitude of the ulnar of both sides

had also greatly declined; the reaction of the ribs and tibial of two legs had also disappeared.

2.F wave: except the ulnar nerve on the right side, all nerves showed no reaction. By nerve conduction testing, this was the impairment (pathological disorder) of the motor nerve.

3.Paralysis had not improved.

Results

Testing of the immunity functions of the case showed IgG being 720 mg/dl (normal value, 608-720 mg/dl), IgA<5.88 mg/dl (normal value, 33-236 mg/dl), IgM being 7.78 mg/dl (normal value, 43-207 mg/dl), CD4/CD8 being 8 (normal value, 1.42-1.90). As IgA, IgM, and CD4/CD8 were all lower than the normal values, the case was decided to be one of immunodeficiency. From school record of the first and second grades, it was noted that the growth of the case was retarding. Family members also mentioned that the case had often been sick since he entered school. It was speculated that immunodeficiency developed when the case was in the first grade.

To decide if AFP was induced by poliovirus, sera of the case were tested to see if they had increased fourfold. The titers of poliovirus neutralization antibodies on the 10^{th} and the 36^{th} days after onset of symptoms were tested to find 16 of poliovirus-1, 32 of poliovirus-2, 8 of poliovirus-3 on the 10^{th} day, and 8 of poliovirus-1, 8 of poliovirus-2, and <8 of poliovirus-3 on the 36^{th} day (Table 4); the titers had declined. The injection of IVIG by the Chang Gung Hospital for suspected serious enterovirus infection to result in interference with serum antibody values could be the reason. Antibody titers could not be used for judgment.

Poliovirus strains have 742 nucleic acids at the 5' non-coding region

primarily for the control of the cloning and expression of genes. Their internal ribosome entry sites (IRES) (Figure 1) are between nucleic acids 130 and 600. The 5' non-coding region is made up by six domains in a stem-loop structure. Partial deletion at the 5' end, before the 130th and after the 600th nucleic acids, will have no impact. Nine poliovirus-1 strains were isolated at different times. The 26th nucleic acid in domain I (D18, except the poliovirus-1 isolated on the 18th day after onset) changed from guanine (G) to adenine (A). The 344th nucleic acid in domain IV (except D18) changed from U to C; the 355th nucleic acid from U to C; the 480th nucleic acid in stem-loop V changed from U to C; the 355th nucleic acid from U to C; the 480th nucleic acid in the stem-loop V changed from G to A, and the neurovirulence had thus increased[15,16] (Table 5). The 742 nucleic acid sequences in the 5' non-coding region had, except D17 strain, a deletion of 8 nucleic acids at the 667th position; and D5 at the 667th and 695th positions had a deletion of 8 and 22 nucleic acids respectively (Figure 2). Nucleic acids G26A, U355C and G480A had already mutated to something similar to the Mahoney strain. The WHO definitions are that the difference between VP1 gene sequence of VDPV and Sabin is between 1 to 15%; and the difference of OPV-like poliovirus and Sabin is within 1%. The 906 nucleic acids in the VP1 region of the eight fecal specimens of the case, when compared with the nucleic acids of the VP1 of the vaccine strain, increased by the time of collection, from 22 differences (2.43%) to 32 different nucleic acids (3.53%) (Table 1). No poliovirus but Echo virus type 7 was isolated in specimens collected after July 29, 2002, speculating that poliovirus disappeared between March and July. Since July, three fecal specimens and throat swabs have been collected every two months to make sure the case is not discharging poliovirus-1 anymore. The polioviruses type 1 isolated from the throat swabs collected on the 5th day after onset and fecal specimens collected on the 17th day after onset, showed a difference of 0.33%

of their VP1 gene sequences. By comparing with the gene mutation of viruses isolated from other fecal specimens, the virus strains of the throat swabs had mutated to a lineage different from that of the fecal specimens, due possibly to the pressure of different selections in different tissues.

The case was inoculated OPV on March 25, May 27, and July 29 of 1993, October 13, 1994, and September 20, 1999, upon entering school (Figure 3). Reports have shown that the annual difference of changes of VP1 is about 1%. The last virus isolated from the case (March 12, 2002) was 3.53% different from the vaccine strain. It was speculated that problems arose after the case took the last dose of OPV (September 20, 1999). The time points corresponded. The VDPV of the case should be vaccine-derived, and not an infection from other persons or the environment.

Discussion

A case of VAPP (vaccine-associated paralytic poliomyelitis), born in 1964, was reported in the US. At age 12, the case was admitted to hospital for infiltrative pneumonia, and was diagnosed CVID (common variable immunodeficiency syndrome) from the immunoglobulin value in serum. At age 16, the case developed fever, diarrhea and later flaccid, and became seriously paralytic and developed poliomyelitis four days later requiring respirator support. In the course of treatment, the case developed pneumonia and urinary tract infection. He had to depend on respirator for the period till his death (1981-October 1990). The case had never contacted any polio patients or vaccinated persons; and he had never been to any epidemic area. He had three doses of inactivated poliovirus vaccine at the age of 3, 4 and 5 months, and four

doses of OPV at 3 years, 3 years and 2 months, 5 years and 10 years[10]. Fecal specimens were collected on the 11th, 23rd, 48th, 126th, 159th and 200th days after onset. Poliovirus-1 was isolated in the specimens. In the specimens collected on the 11th day, two subtypes of poliovirus were isolated. Their VP1 genes were about 10% different from the vaccine strains. Only one subtype was isolated in specimens collected after the 11th day of onset. It could be noted from the VP1 sequences that gene sequences mutated step by step, at a speed of 1.1% per year, to continue to excrete viruses. Estimates were that the virus could survive in human body for as long as seven years.

In the United Kingdom, a case[12], a 20-year old woman, had had repeated infection since 1957 (15 years old). The IgG tested then at the hospital was 400 mg/ml; it went down in the next two years to 200 mg/ml and 120 mg/ml. Before the injection of immunoglobulin, the titers of the polio neutralization antibodies I, II and III were all 1:16; the titer in 1958 was 1:4. In December 1961, the case was given OPV-I; the IgG then was 500 mg/ml, and the titers of the polio neutralization antibodies I, II and III were 1:8, 1:8 and 1:32; and no type 1 virus was detected. In January 1962, one dose of type 3 vaccine was given. In December 1962, poliovirus-3 was excreted for 21 months till October 1963. In the meantime, polio type 2 vaccine was given, hoping to interfere the excretion of type 3 virus. It was not successful, and no type 2 virus was detected. The above-mentioned two cases were cases of immunodeficiency that had, after taking OPV, excreted viruses for long time. Vaccine strains then mutated by time to regain pathogenic factors to cause infection in the cases. This could happen only in developed countries of higher medical care standards; because children of immunodeficiency in developing countries would have died of other infections, and the OPV taken will have not yet become pathogenic before the children die.

For the likely sequelae of iVDPV, many developed countries are using IPV instead. Chances of this disorder should be very small in the future.

Albert Sabin developed OPV by attenuating polioviruses of three virulent sera types. The mutated part of the vaccine could reduce the neurovirulence of poliovirus. Poliovirus-1 has one mutation at the 5' non-coding region (5'-UTR); two amino acids are replaced at VP1; and one each amino acid is replaced at VP3 and VP4. Poliovirus-2 has one mutation at the 5' non-coding region (5'-UTR); and one amino acid is replaced at VP1. Poliovirus-3 has one mutation at the 5' non-coding region (5'-UTR); and one amino acid is replaced at VP4. OPV clones in the small intestines of human beings and could mutate back to wild strain. Chances in normal persons are, however, as low as one-millionths.

There were outbreaks of poliomyelitis in Dominican Republic and Haiti in 2000-2001[9]. In Dominica, there were 13 confirmed cases; and in Haiti, there were eight confirmed cases, with two deaths. These outbreaks were considered to be infection of vaccine-derived poliovirus-1 strain. In 1998-1999, children were given one dose of OPV. Those who became sick either did not take the vaccine or the vaccination was incomplete (did not complete three doses of OPV). The vaccination coverage rate in community was only 7-40%. VP1 sequencing of virus showed 97% of similarity with the sabin type-1 vaccine. The neuroparalytic symptoms were similar to those induced by the wild strain.

In 2002, the AFP monitoring system of Madagascar detected a clustering of poliomyelitis[13]. By virus culturing, vaccine-derived poliovirus-2 strains were isolated. There were four patients. The pathogenic agent was considered to be the cVDPV (circulating vaccine-derived poliovirus) in the community. Investigation showed that in the year 2000, only 37% of children

under one year had had three doses of OPV. In 2001, the non-polio AFP rate of children under 15 years was 0.4 per 100,000 population, lower than the target of 1 set out by the WHO.

In October 2000, the WHO declared the eradication of poliomyelitis from the Western Pacific Region. By 2005, the goal of the global eradication of poliomyelitis would be attained. After the global eradication, vaccination can be terminated, and resources can be used for the control of other communicable diseases. Patients of immunodeficiency will, however, continue to excrete poliovirus for as long as ten years. If vaccination is terminated after the global eradication of poliomyelitis, iVDPV could possibly induce cVDPV-like infection. The termination of OPV vaccination may have to wait a while. The cost-benefit and feasibility of shifting from OPV to IPV after the global eradication should be assessed carefully. Findings of the present study are meaningful in that they can serve as a reference in the formation of vaccine strategies after the eradication of poliomyelitis.

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Table 1 Specimen Collection, Virus Isolation, and VP1 Gene Difference with Vaccine Strain of iVDPP Case

No	Date of Collection	Days from Onset	Specimen	Virus Isolated	Different nt of VP1(960nt)(%)
1	2001/04/14	5	TH	Polio-1	25(2.76)
2	2001/04/16	17	Stool	Polio-1	22(2.43)
3	2001/04/27	18	Stool	Polio-1	22(2.43)
4	2001/05/31	52	Stool	Polio-1	25(2.76)
5	2001/06/02	54	Stool	Polio-1	25(2.76)
6	2001/10/05	179	Stool	Polio-1	27(2.98)
7	2001/11/19	224	Stool	Polio-1	29(3.20)
8	2001/12/26	261	Stool	Polio-1	29(3.20)
9	2002/03/12	337	Stool	Polio-1	32(3.53)

Note：Date of onset was 2001/04/09

Table 2. Non-Coding Region, VP1 as Gene Sequencing Primer

VP1 primer	Q8: 5'AAGAGGTCTCTATTCCACAT 3' Y7: 5'GGTTTTGTGTCAGCGTGTAATGA 3'
5'non-cording region primer	EV1: 5'CAAGCACTTCTGTTTCCCCGG 3' EV2: 5'ATTGTCAACCATAAGCAGCCA 3'

Table 3. Virus Isolation in Contacts of Case

Contacts	No	Poliovirus negative	Other viruses
Class 3, Grade 2	33	33	2(adenovirus)
After-school class	11	11	0
Community	15	15	0
Siblings	3	3	1(adenovirus)

Table 4. Antibody Titers of Poliomyelitis on the 10th and 36th Day after Onset of Case

Days after onset	Poliovirus-1	Poliovirus-2	Poliovirus-3
10	16	32	8
36	8	8	＜8

Table 5. Differences of Virus Strains Isolated at Different Times and Vaccine Strains and Mahoney at 5'non-Coding Region

Virus	Nucleic Acid Position			
	26	344	355	480
Sabin-1	G	T	T	G
D5	A	C	C	A
D17	A	C	C	A
D18	G	T	C	A
D52	A	C	C	A
D54	A	C	C	A
D179	A	C	C	A
D224	A	C	C	A
D261	A	C	C	A
D337	A	C	C	A
Mahoney	A	T	C	A

Figure 1. Stem-Loop Structure of Poliovirus at 5' Non-Coding Region

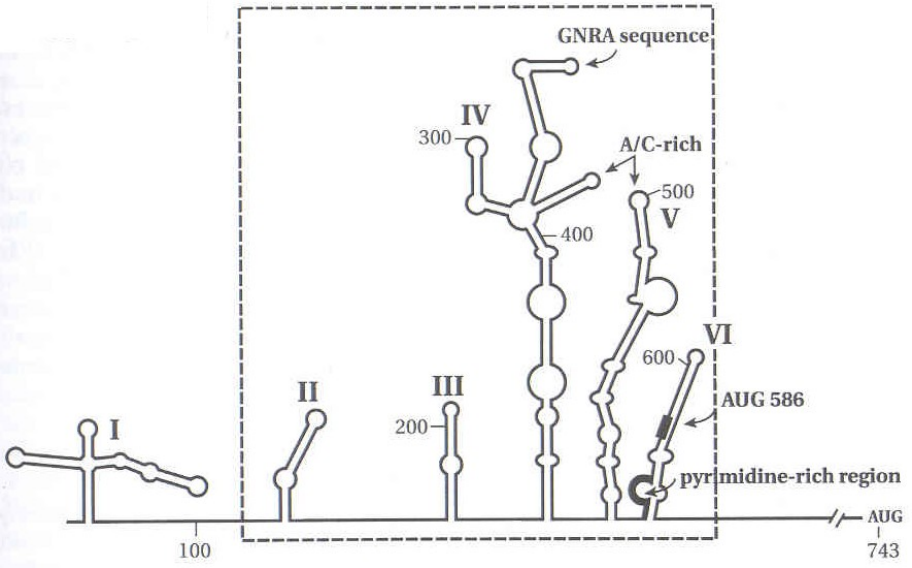

Figure 2. Sequences of Nucleic Acid Deletion at 5' Non-Coding Region

Sabin-1	**TGCTG**	**GATTCGCTCC**	**ATTGAGTGTG**	**TTTACTCTAA**	**GTACAATTTC**	**AACAGTTA**
D5	**TNNNN**	**NNNNCGCTCC**	**ATTGAGTGTG**	**TTTANNNNNN**	**NNNNNNNNNN**	**NNNNNNTA**
D17	**TGCTG**	**GATTCGCTCC**	**ATTGAGTGTG**	**TTTATTCTAA**	**GTACAATTTC**	**AACAGTTA**
D18	**TNNNN**	**NNNN CGCTCC**	**ATTGAGTGTG**	**TTTATTCTAA**	**GTACAATTTC**	**AACAGTTA**
D52	**TNNNN**	**NNNN CGCTCC**	**ATTGAGTGTG**	**TTTATTTTAA**	**GTACAATTTC**	**AACAGTTA**
D54	**TNNNN**	**NNNN CGCTCC**	**ATTGAGTGTG**	**TTTATTTTAA**	**GTACAATTTC**	**AACAGTTA**
D179	**TNNNN**	**NNNN CGCTCC**	**ATTGAGTGTG**	**TTTATTTTAA**	**GTACAATTTC**	**AACAGTTA**
D224	**TNNNN**	**NNNN CGCTCC**	**ATTGAGTGTG**	**TTTATTTTAA**	**GTACAATTTC**	**AACAGTTA**
D261	**TNNNN**	**NNNN CGCTCC**	**ATTGAGTGTG**	**TTTATTTTAA**	**GTACAATTTC**	**AACAGTTA**
D337	**TNNNN**	**NNNN CGCTCC**	**ATTGAGTGTG**	**TTTATTTTAA**	**GTACAATTTC**	**AACAGTTA**