EPIDEMIOLOGICAL STUDY ON POLIOVIRUS ISOLATES FROM PATIENTS WITH ACUTE FLACCID PARALYSIS IN SHANDONG PROVINCE IN CHINA

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SUMMARY: In five years from 1988 to 1992, 51 polioviruses were isolated from patients with acute flaccid paralysis in Shandong province in China. Of the 51 poliovirus isolates, 17 were type 1, 18 type 2 and 16 type 3. Twelve type 1 viruses isolated during the period from 1988 to 1990 were shown to be wild strains by serology and the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods. These wild strains were classified into three groups by PCR-RFLP. The other type 1 isolates were Sabin-like strains. No wild strain was isolated in 1991 or 1992. All isolates of types 2 and 3 during the five years were Sabin-like strains. These data suggest that wild strains of polioviruses will soon be eradicated in Shandong province.
INTRODUCTION

A plan of action for the global eradication of poliomyelitis by the year 2000 was endorsed by the World Health Organization in 1989 (1). In China, the big epidemics broke out during the period from 1988 to 1990. To clarify the status of the epidemics, poliovirus isolates from patients with acute flaccid paralysis (AFP) were examined for their characters by serological methods and PCR-RFLP. Here we discuss the situation of poliomyelitis in China.

MATERIALS AND METHODS

Virus isolation: HEp-2 and RD cells were used for virus isolation. Fecal samples were collected from children suffering from AFP in Shandong province. A 10% fecal suspension in phosphate buffered saline was prepared and inoculated into two cell lines.

Virus identification: All isolates were identified with poliovirus antiserum pools and antisera to other enteroviruses.

Intratypic differentiation of poliovirus isolates: Intratypic differentiation was carried out by serological methods [the McBride method (2) or monoclonal antibody method] and the PCR-RFLP method. PCR-RFLP method was done by the following procedures. The genomic variability of poliovirus isolates was analyzed by the restriction fragment length polymorphism assay according to the method of Balanant et al (3). The purified RNA was reverse-transcribed with cloned Moloney murine leukemia virus reverse transcriptase (Perkin-Elmer Cetus) Instrument, Norwalk, Conn. and PCR-amplified with Taq DNA polymerase (Perkin-Elmer Cetus). The primers common to all poliovirus types were used. The downstream primer has the sequence 5'-GAATTCCATGTCAAATGA and the upstream primer the sequence 5'-TTTGTGTCAGCGTAATGA. The fragment was 480-nucleotide sequence of poliovirus genome cording the N-terminal half of the capsid protein VP1 including antigenic site 1 (nucleotide 2402-2881).

RESULTS

Virus Isolation

Sample collection was incomplete during the period from 1988 to 1990. In 1991 and 1992, samples were collected from most patients. Isolation was success-
ful in 35% of the patient samples in each year. The low isolation rate was caused probably by inappropriate time of collection, incomplete cold chain and by difficulty in quick transportation of samples to the laboratory. During the five years, 51 polioviruses were isolated. Of the 51 poliovirus isolates, 17 were type 1, 18 type 2 and the other 16 type 3 (Table I).

### Table I. Identification of poliovirus isolates

<table>
<thead>
<tr>
<th>Year</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
<th>Number of poliomyelitis cases</th>
</tr>
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<tr>
<td>1988</td>
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<td>1</td>
<td>1</td>
<td>226</td>
</tr>
<tr>
<td>1989</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>484</td>
</tr>
<tr>
<td>1990</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>284</td>
</tr>
<tr>
<td>1991</td>
<td>2</td>
<td>7</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>1992</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>18</td>
<td>16</td>
<td>1114</td>
</tr>
</tbody>
</table>

### Table II. Intratypic differentiation of poliovirus isolates

<table>
<thead>
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<th>Year</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
<tbody>
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<td>S</td>
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<td>S</td>
</tr>
<tr>
<td>1988</td>
<td>1</td>
<td>3</td>
<td>1</td>
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<tr>
<td>1992</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>12</td>
<td>18</td>
</tr>
</tbody>
</table>

Fig. 1. The RPLF patterns of wild poliovirus type 1 isolates in Shan- 
dong province related to Sabin 1 vaccine strain. The genomic viral 
RNA was extracted, reverse-transcribed, and amplified. Each of the 
resulting DNA fragments (approximately 480 bp) was separately digest-
ed with Dde I, Hae III, or Hpa II. The digestion products were run on 
agarose gel in parallel with molecular-weight markers ($\phi \times 174$/Hae 
III) and an uncut (UC) DNA fragment derived from Sabin 1 strain.

**Intratypic Differentiation of Isolates**

The results by PCR-RFLP perfectly agreed with those by the McBride and 
monoclonal methods (data not shown).

The 51 isolates were examined for their characteristics. Intratypic differen-
tiation was carried out with monoclonal antibodies and by the PCR-RFLP tech-
nique. As shown in Table II, wild strains were isolated during the period from
1988 to 1990 and all of them were type 1. No wild strain was isolated in 1991 or 1992. Types 2 and 3 isolates during the five years were all Sabin-like strains. This means that type 1 wild strains were causative agents of the epidemic of poliomyelitis in Shandong province during the three years from 1988 to 1990. On the other hand, AFPs caused by type 2 and 3 polioviruses were probably the contact or vaccine-associated cases.

The RFLP patterns of wild strains of type 1 are shown in Fig. 1. From these profiles, the wild type 1 strains of Shandong were classified into three groups.

DISCUSSION

In Shandong province in China, 70 and 71 cases were confirmed as poliomyelitis in 1986 and 1987, respectively. Poliomyelitis epidemics broke out in 1988 and continued till 1990. The confirmed cases in 1988, 1989 and 1990 were 226, 484 and 284, respectively. The number of patients was reduced to 25 in 1992 by the Expanded Programme on Immunization activity, including the OPV mass immunization campaigns.

Polioviruses were isolated from patients and differentiated intertypically and intratypically. During the five years, 12 wild strains were isolated and the other 39 isolates were Sabin-like strains. When the wild strain ceased to be detected in 1991, the number of patients with paralysis was reduced to one-twentieth that in 1989. Therefore these wild strains are supposed to be causative agents of the polio-epidemics in Shandong province.

The Sabin-like strains are supposed to be causative agents of vaccine-associated cases. If so, we have to discuss why Sabin-like strains were so frequently isolated. In 1992, about 20 million doses of oral polio vaccine were administered to children in Shandong province and the number of poliomyelitis cases was 25. Therefore, we estimate that vaccine-associated cases occurred at a rate of one case per 800,000 doses. Nkowane et al. (4) and de Quadros et al. (5) reported that the average ratio of the risk of vaccine-associated case by OPV was one per 2.6 million doses. The WHO also reported that one vaccine-associated case was calculated to occur every one million doses at the first vaccination of OPV (6). The ratio of the risk by OPV in China is almost the same as that in the United States or that reported by WHO. In Japan, from 1964 to 1969, 13-17 pa
Patients of poliomyelitis appeared annually. The incidence was 1 per 200,000-100,000 distributed doses. During this 6-year period, only one wild type appeared among 51 isolates. Therefore, the situation is similar between Japan 1964-1969 and Shandong province 1990-1992 with respect to the incidence of vaccine strains isolated. As the vaccine program was a great success in Japan, it is safe to conclude that the vaccine program in Shandong province is on the right track. The characterization of Sabin-like strains isolated from patients is under way.

By PCR-RFLP, the 12 wild isolates of type 1 were classified into three groups, group-1, -2 and -3. Seven of group-1 were isolated during the period from 1988 to 1990, three of group-2 in 1988 and 1989, and two of group-3 in 1990 only. The patterns after digestion with Hpa II were the same among the three groups. The pattern of group-1 after Hae III digestion was identical with that of group-3. From all these observations, it is speculated that group-2 is an original strain and that group-1 is derived from group-2 and group-3 from group-1.

The eradication of poliomyelitis will soon be achieved in Shandong province.

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REFERENCES

