Characterisation of vaccine-derived polioviruses isolated from sewage and river water in Japan

Hiromu Yoshida, Hitoshi Horie, Kumiko Matsuura, Tatsuo Miyamura

Summary

Background A nucleotide change from U to C at position 472 in the 5' non-coding region of the type 3 poliovirus is associated with increased neurovirulence. Moreover, the proportion of type 3 polioviruses containing this mutation (472-C revertants) correlates with the neurovirulence of a particular sample. We used mutant analysis by PCR and restriction-enzyme cleavage (MAPREC) to estimate the neurovirulence of environmental samples obtained from Toyama prefecture, Japan.

Methods Sewage and river water were collected between October, 1993, and September, 1995, and concentrated samples were inoculated into three different cell types. Isolated type 3 viruses were analysed to determine whether they were derived from the live oral poliovirus vaccine strain; they were then tested for neurovirulence by MAPREC.

Results 29 type 3 strains were isolated—of which were vaccine-derived. 16 (55%) comprised between 2% and 91% 472-C revertants by MAPREC and were expected to have high neurovirulence. The remaining strains included less than 0.25% revertants, and were regarded as attenuated viruses. Both types were isolated about 3 months after routine oral poliovirus vaccine administrations in May and October. Three strains isolated from river water were of the virulent type.

Interpretation Our results emphasise that there is an environmental risk of vaccine-associated paralytic poliomyelitis as long as live oral poliovirus vaccine is not replaced by inactivated polio vaccine.


Introduction

WHO adopted a target of the year 2000 for eradication of poliomyelitis in the western Pacific region. No wild strain has been isolated from paralytic patients in the western Pacific region since March, 1997, and an imported wild strain was the cause of the 1999 case in China. However, to eradicate poliomyelitis, we must address the fact that as long as immunisation with live oral poliovirus vaccine continues, the risk of vaccine-associated paralytic poliomyelitis will still exist. In 2000, the USA started to replace oral poliovirus vaccine by the inactivated poliovaccine to reduce this risk.

Some have reported that when position 472 in the 5' non-coding region of type 3 poliovirus mutates from U to C, the neurovirulence increases. Sequencing analysis at position 472 of the genome of excreted viruses showed that the substitution occurred gradually while the virus replicated in the human gut after administration of oral poliovirus vaccine. Moreover, the study (which involved mutant analysis by PCR and restriction-enzyme cleavage [MAPREC]—a technique developed by Chumakov and colleagues as a quality-control test for oral poliovirus vaccine) showed that the proportion of type 3 viruses containing C at position 472 (472-C revertants) correlated with neurovirulence in monkeys. MAPREC is useful not only as a quality-control test for oral poliovirus vaccine but also for laboratory surveillance based on virological diagnosis.

The study and characterisation of vaccine strains excreted from the human gut and in the environment is important for the eradication of poliomyelitis and vaccine-associated paralytic poliomyelitis. Oral poliovirus vaccine is given twice annually (in May and October) in Toyama prefecture, Japan. Environmental surveillance in sewage and river water was carried out from October, 1993, to September, 1995. As a result, 16 type 1, 31 type 2, and 31 type 3 polioviruses were isolated. In the present study, we analysed the isolated type 3 viruses by MAPREC to estimate their neurovirulence.

Methods

Sewage and river water (1 L and 700–800 mL, respectively) were collected twice monthly at the sewage plant and the river-monitoring points in Toyama, and were concentrated by the filter adsorption/elution method. The concentrated specimens were inoculated into rhabdomyosarcoma, primary monkey kidney, or Vero cells, and incubated at 36°C. Type 3 isolates identified by polio pooled serum were used for further study.

The proportion of 472-C revertants was examined by the method described by Chumakov and colleagues. Amplified cDNA containing a virulent base C was identified by restriction-fragment-length polymorphism (RFLP) analysis with MspI. The F313 type 3 strain was used as a reference strain for the attenuate phenotype, and the Leon strain was used as the reference virulent-

Department of Virology II, National Institute of Infectious Diseases, Gakuen 4-7-1, Musashimurayama, Tokyo, 208-0313, Japan (H Yoshida msdsc, T Miyamura rr); Japan Poliomyelitis Research Institute (H Horie rr); and Department of Virology, Toyama Institute of Health (K Matsuura ss)

Correspondence to: Dr Hiromu Yoshida
(e-mail: hyoshida@nih.go.jp)
other three strains were isolated from the river Sembo (two strains) and the river Itachi (one strain). The results of intratypic differentiation by both methods showed that all isolates were derived from vaccine strains (data not shown).

Analysis of isolates by MAPREC revealed two different groups on the basis of the proportion of 472-C revertants they contained (figure 1). One group (16 strains [55%]) contained 2-9% 472-C revertants, which is much higher than the stipulated 1% above which type 3 vaccine viruses are deemed unsafe by the monkey neurovirulence test.27-29 The proportion of 472-C revertants in the other group (13 strains) was less than 0.29%, which is close to the reference value for the F313 strain.

The proportion of 472-C revertants in isolates S9-2, S25-1, and I42-1, which were isolated from river water in February and October, 1994, and in June, 1995, were 90-7%, 90-0%, and 88-1%, respectively. The number of type 3 polioviruses isolated during each month of surveillance are shown in figure 2.

Discussion
Poliovirus virulence has been measured by the monkey neurovirulence test, the T or d-marker test, and by direct sequencing.30 A sequencing study showed that in the 5’ non-coding region, a change from G to A at position 480 in type 1 polioviruses, or from A to G at position 481 in type 2 viruses, or from U to C at position 472 in type 3 viruses, influenced neurovirulence.31-33 In-vitro and in-vivo studies revealed that these substitutions occurred during replication in the human gut.34 Moreover, MAPREC revealed that the proportion of 472-C revertants in the type 3 poliovirus population was correlated to the histological lesion score in the monkey neurovirulence test. For this reason, MAPREC is applied as the quality-control test for oral poliomyelitis vaccine. In the present study, the neurovirulence of type 3 polioviruses isolated from the environment was examined by MAPREC.

In Japan, the last wild-strain poliovirus was isolated from poliomyelitis patients in 1980. However, it is important to analyse viruses isolated from the environment to assess vaccine efficacy after the elimination of wild type. Vaccine-derived virus strains isolated from the environment were reported to have slight mutations in the VP-1 region of the viral genome.29-31 In our previous study, type 3 poliovirus strains from sewage and river water also had slight mutations, which amounted to less than 1.4% nucleotide divergence from the vaccine strain.32 Since vaccine strains were thought to have replaced wild type in the environment of Japan, MAPREC was expected to be helpful in environmental surveillance for vaccine-derived strains. In this study, the environmental strains were classified by MAPREC into virulent and attenuate phenotypes.

Some have reported that the proportion of revertants in the virus population is influenced by the passage number, the temperature at which the virus was grown, and the multiplicity of infection of the virus.35-37 In this study, three kinds of cell lines were used for virus isolation, because it was not always possible to use only one cell line owing to the condition of the specimen. Although a given cell line could have influenced the rate of selection for revertants in the virus population, the influence would have been small for viruses isolated from the environment. Our data showed that the passage number did not always correlate with the proportion of 472-C revertants isolated. For example, after the second passage in Vero cells at 36°C, 13 strains were isolated; six strains were attenuated type, and seven virulent type. In the third passage under the same conditions, four strains were isolated; one was attenuated type, the other three virulent types.
Three virulent types were isolated from river water in the Toyama prefecture in February, 1994, October, 1994, and June, 1995. Oral poliomyelitis vaccine in this region was given in May and October. For this reason, the two cases isolated in October, 1994, and June, 1995, were regarded as polioviruses excreted from human beings after administration of oral poliomyelitis vaccine. Polioviruses isolated in February, 1994, had three possible sources: the virus was (1) continuously excreted from within the human population, (2) living in the water, or (3) circulating in the human community. In the field trial of oral poliomyelitis vaccine, poliovirus was found to be excreted from healthy children for 2–3 months after vaccine administration.\(^\text{13,16,17}\)

It was shown, however, that the period over which poliovirus was excreted from individuals previously immunised with oral poliomyelitis vaccine or infected naturally was shorter than the excretion period of susceptible individuals.\(^\text{13,14,16}\) Therefore, the attenuated isolates from the environmental samples might have been excreted by a previously immunised group, and the virulent isolates from a susceptible group. It is not clear to which case the environmental isolates described here should be attributed.

Since there is little chance that individuals come into direct contact with raw sewage, the risk of infection by this route is very low. However, access to the river is easy for many individuals and susceptible individuals should be regarded as at greater risk of infection from river water. Moreover, although it is possible to eliminate wild-type poliovirus from the human community and environment, as long as oral poliomyelitis vaccine is not replaced by inactivated polio vaccine, it will be difficult to eradicate poliomyelitis completely.

**Contributors**

Hiroshi Yoshida was responsible for the conception of the study, and for MAPREC analysis. Hitoshi Horie provided MAPREC expertise. Kuniako Matsuura contributed to virus preparation and in vitro differentiation. Tatsuo Miyamura contributed to the epidemiological analysis.

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