Application of traditional herbal medicines to suppress the reversion of polio vaccine viruses to the neurovirulent genotype

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Background The oral poliovirus vaccine (OPV) is regarded as one of the most effective and safest vaccines. However, some paralytic cases have been reported among vaccinees and individuals who have been in contact with vaccinees, because vaccine viruses undergo neurovirulent reversion during repeated replication in the alimentary tract. Moreover, the revertants are excreted with the feces into the environment, and the viruses have caused epidemics of poliomyelitis in the world.

Objective We have reported that Ninjin'yoeito suppressed the reversion of polio vaccine viruses. In this study, to clarify the herbal constituent which takes part in the suppressive effect, we investigated the effect of Ninjinto, which is composed of simpler herbal constituents than Ninjin'yoeito, and of the individual herbal constituents in Ninjinto.

Methods Polio vaccine viruses were serially passaged three times in the presence of herbal medicines in cells derived from the human alimentary tract. The reversion of the passaged viruses was analyzed by the MAPREC and non-RI-modified MAPREC method, designed to estimate the ratio of revertants in a virus population.

Results It was shown that Ninjinto suppressed the reversion of polio vaccine viruses. Although Ginseng Radix and Glycyrrhizae Radix (common herbal constituents in Ninjinto and Ninjin'yoeito) did not show the suppressive effect, the mixture of both herbal constituents was a little inhibitory in regard to the reversion of the viruses.

Conclusion It is quite likely that the suppressive effect on reversion is not a simple process. A complicated interaction of some components of the herbal constituents might take part in the effect. It is expected that traditional herbal medicines like Ninjinto and Ninjin'yoeito will contribute to solving the problems of OPV though further analyses of the herbal constituents and their components are necessary to clarify the mechanism of the suppressive effect.

Key words poliovirus, oral poliovirus vaccine (OPV), neurovirulent reversion, Ninjinto, Ninjin'yoeito
Introduction

The trivalent live oral poliovirus vaccine (OPV) is constituted of attenuated Sabin strains of three serotypes. It is regarded as one of the most effective and safest vaccines in current use, and it has been used as a major tool for the global polio eradication program of the World Health Organization (WHO). However, some paralytic cases (vaccine-associated paralytic poliomyelitis: VAPP) have been reported among vaccinees and individuals who have been in contact with vaccinees (WHO Consultative Group; 1982, Nkowane et al.; 1987). Such cases are thought to have been caused by revertants of vaccine viruses that have mutated during repeated replication in the alimentary tract of vaccinees (Evans et al.; 1985). Moreover, as long as OPV is in use, the revertants (vaccine-derived poliovirus: VDPV) continue to be excreted with the feces into the environment, and the VDPV has caused epidemics of poliomyelitis worldwide (Centers for Diseases Control and Prevention; 2006). The VAPP and VDPV problems are obstacles for the achievement of the polio eradication program. New approaches for the suppression of the reversion of polio vaccine viruses to the neurovirulent genotype are urgently needed to solve these problems.

Recently, the genome structures of a number of attenuated and virulent strains of the three types of poliovirus have been analyzed. It was demonstrated that neurovirulence increases when the following changes take place in the base positions of the vaccine viruses: in the case of the type 1 virus, position 480 and 525 in the 5' non-coding region changed respectively from G to A (Kawamura et al.; 1989, Horie et al.; 1994) and from U to C (Muzychenko et al.; 1991) ; for type 2, position 481 changed from A to G (Pollard et al.; 1989), and for type 3, position 472 changed from U to C (Evans et al.; 1985). Furthermore, Chumakov et al. (1991, 1992) developed the MAPREC (mutant analysis by PCR and restriction enzyme cleavage) method, and Horie et al. (1998) established a non-RI-modified MAPREC method to estimate the ratio of revertants in a virus population that is correlated with neurovirulence in monkeys (Rezapkin et al.; 1994, Taffs et al.; 1995, Chumakov et al.; 1999). MAPREC is a very sensitive method, and is useful for monitoring the reversion of the polio vaccine virus genome.

Accumulation of reversion of the poliovirus genome is easily observed by passaging it in cultivation cells (Chumakov et al.; 1991, 1994, Horie et al.; 1999, 2001, 2008). It has been reported that reversion was promoted under cell conditioning like overproliferation (Chumakov et al.; 1994). It is considered that the activity of such overproliferous cells is low, that is, there is a possibility that reversion is suppressed when cell activity is maintained under high conditioning. Ninjinto (Ren-Shen-Tang in Chinese) and Ninjin'yoeito (Ren-Shen-Yang-Rong-Tang in Chinese) are traditional herbal medicines (Japanese name: Kampo) which are suitable for long-term oral administration with few adverse reactions. Ninjinto and Ninjin'yoeito have received attention as immune activators, and clinical applications have been attempted for the treatment of fatigue and after long-term illnesses. It is expected that such traditional herbal medicines have activation effects on individuals and cells, and that they suppress the reversion of polio vaccine viruses to the neuroviral genotype. Actually, we have reported that Ninjin'yoeito had a suppressive effect on the reversion of the viruses (Horie et al.; 2007).

In this paper, we investigated the suppressive effect of Ninjinto (composed of fewer herbal constituents compared with Ninjin'yoeito) on the reversion of polio vaccine viruses, and which of the herbal constituents take part in the suppressive effect in cultivation cells derived from the human alimentary tract by using the MAPREC and non-RI MAPREC methods.

Materials and Methods

1) Traditional herbal medicines

Spray-dried extract preparations of Ninjinto (TJ-32), Ninjin'yoeito (TJ-108), Ginseng Radix and Glycyrrhizae Radix were kindly supplied by Tsumura & Co. The herbal constituents and contents of TJ-32 and TJ-108 are described in Table 1. Three-dimensional HPLC (3-D-HPLC) analyses were carried out to find out the broad chemical profiles of TJ-32 and TJ-108. The spray-dried
Table 1  Herbal constituents and contents of traditional herbal medicines

<table>
<thead>
<tr>
<th>Herbs</th>
<th>TJ-108</th>
<th>TJ-32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angelicae Radix</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>Astragalii Radix</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Atractyloidis Rhizoma</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>Atractyloids Lanceae Rhizoma</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>Aurantii Nobilis Pericarpium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cynnamomi Cortex</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>Ginseng Radix</td>
<td>9.7</td>
<td>25.0</td>
</tr>
<tr>
<td>Glycyrrhizae Radix</td>
<td>3.2</td>
<td>25.0</td>
</tr>
<tr>
<td>Hoelen</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>Paeniae Radix</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>Polygalae Radix</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>Rehmanniace Radix</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>Schisandrae Fructus</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Zingiberis Siccatum Rhizoma</td>
<td>25.0</td>
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</tr>
</tbody>
</table>

extract preparations of both medicines with their 3-D-HPLC profiles showing in Figs 1 and 2 were used for this study.

2) Cell and virus growth

Caco-2 cells (RCB0988) derived from human colon carcinoma were obtained from Riken Cell Bank. The cells were propagated in the Minimum Essential Medium (MEM, Invitrogen Gibco) containing 20% fetal bovine serum at a temperature of 37°C. Cell growth was determined in the presence of three concentrations (0.25, 0.5 or 1.0 mg/mL) of TJ-32 or TJ-108, four concentrations (0.5, 1.0, 2.0 or 4.0 mg/mL) of Ginseng Radix, Glycyrrhizae Radix or a mixture of Ginseng Radix and Glycyrrhizae Radix for up to 10 days. Each initial culture had 2 x 10⁴ cells/mL, and viability was determined by trypan blue exclusion.

Poliovirus Sabin type 1 and 3 attenuated vaccine strains were used in this study. Before inoculation of the viruses to the Caco-2 cells, the cells were cultured in the presence of 0.5mg/mL TJ-32 or TJ-108, 2 mg/mL Ginseng Radix, Glycyrrhizae Radix or a mixture of Ginseng Radix and Glycyrrhizae Radix in MEM with 2% fetal bovine serum in a cell culture flask (cultivation area: 25 cm²) at 37°C for 5 days. The viruses were serially passaged three times in confluent cultures of the cells which contained the herbal medicines described above in 6 mL/flask of Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen Gibco) with 2% fetal bovine serum at 37°C. The virus inoculation methods are as follows. The viruses were diluted at 1:100 with DMEM containing 2% fetal bovine serum, and 0.2 mL of the diluted virus was inoculated to the cells. From the results of virus titration, multiplicity of infection (m.o.i.) was approximately 10⁻¹ - 10⁻² 50% tissue culture infectious doses (TCID₅₀)/cell. Adsorption of the virus to the cells was performed at 37°C for 30 min. Virus-specific cytopathic effects (CPE) were observed at 20, 30, 44, 48 and 54h post infection. The viruses were harvested when all cells were detached by the CPE.

3) Virus titration

Virus titration was performed in 96-well microtiter plates using HEp-2 cells in the standard manner (World Health Organization; 1995). The CPE was read on day 7 post infection. Measurement was performed five times and the data were
analyzed using Student's t-test. A value of $p<0.05$ was considered statistically significant.

4) MAPREC

Base changes for the type 1 Sabin virus with a virulent base A at position 480 and base C at position 525, and for the type 3 Sabin virus with a virulent base C at position 472 were examined by the MAPREC method or non-RI-modified MAPREC method. MAPREC was performed according to the method of Chumakov et al. (1994) and Rezapkin et al. (1994). Details of the method have been described in our previously reports (Horie et al.; 2007, 2008). Non-RI MAPREC was performed according to the method of Rorie et al. (1998). The MAPREC and non-RI MAPREC methods are identical except for that used for quantifying digested DNA bands at the terminal stage of the analytical procedure.Briefly, after treatment of the amplified DNA product with each restriction enzyme (Alu 1 for 480-A in type 1, ScrF 1 for 525-C in type 1 and Mbo 1 for 472-C in type 3), the digested material was separated by 12% polyacrylamide gel electrophoresis. The gel was stained for 30 min with SYBR Green 1 (Takara Bio Inc.) diluted 20,000-fold with TBE (100mM Tris, 90mM boric acid, 1 mM EDTA, at pH 8.4). The digested DNA bands were detected by irradiating with ultraviolet rays at a wavelength of 250 nm and quantities of fluorescence intensity of high-molecular-weight bands were determined by the use of a VersaDoc 5000 imaging system (Bio-Rad Laboratories).

The virus cultivation (passaging) with herbal medicines was performed three times independently (experiment 1, 2 and 3). Cultured viruses with TJ-32 or TJ-108 in experiment 1 were analyzed by the MAPREC method. Viruses with TJ-32, TJ-108, Ginseng Radix, Glycyrrhizae Radix or a mixture of Ginseng Radix and Glycyrrhizae Radix in experiment 1, 2 and 3 were analyzed by the non-RI MAPREC method. The data of the non-RI method were analyzed using Student's t-test and a value of $p<0.05$ was considered statistically significant.

Results

1) Effects of TJ-32 on proliferation of cells and polio vaccine viruses

Three concentrations of TJ-32 and TJ-108 (0.25, 0.50 and 1.0 mg/mL) and the Caco-2 cells derived from the human alimentary tract were used for the tests. For the concentrations of 0.25 and 0.50 mg/mL TJ-32, an equivalent cell proliferation was exhibited compared with control cells which were not treated with the medicine (Fig. 3). On the contrary, clear inhibition of cell proliferation was observed for the concentration of 1.0 mg/mL. Also, in the case of TJ-108, similar results were obtained. The data on TJ-108 were described in our previous report (Horie et al.; 2007). We therefore decided to use the highest concentrations (0.5 mg/mL) which did not inhibit the proliferation of the cells for the virus cultivation.

Poliovirus Sabin type 1 and 3 vaccine strains were passaged three times using Caco-2 cells cultured with 0.5 mg/mL TJ-32 or TJ-108. Progressive rate of specific CPE of the virus passaged the first time was almost the same as the control viruses which were cultured without medicine in type 1 and 3 viruses. The cultivation period was 48 to 54h (data not shown). In the viruses passaged the second and third times, an inclination for the progressive rate of CPE to become faster was observed including the control viruses, the cultivation period was 44 to 48h (data not shown). Titers of the passaged viruses were measured using the microtiter method (Fig. 4). Although the virus titer increased a little with virus passaging, no significant difference in the titers of the viruses passaged with both medicines was observed compared with those of control viruses, which were not treated with the medicines in the type 1 and 3 viruses (Fig. 4A, B). TJ-32 and TJ-108 did not
Suppression of poliovirus reversion by traditional medicines

2) Suppressive effect of TJ-32 on reversion of polio vaccine viruses

In passaged viruses with TJ-32 and TJ-108, the proportions of type 1 viruses containing A at position 480 (480-A revertants), C at position 525 (525-C revertants), and type 3 viruses containing C at position 472 (472-C revertants) were analyzed by the MAPREC method. Accumulations of the 480-A and 525-C reversions in type 1 and the 472-C reversion in the type 3 vaccine viruses during passaging with TJ-32 and TJ-108 are shown in Fig. 5. The suppressive effect of TJ-108 on the reversion of polio vaccine viruses was reported in our previous study (Horie et al.; 2007). It was demonstrated that TJ-32, which is composed of fewer herbal constituents than TJ-108, suppressed the reversion compared with the control virus in the type 1 and 3 viruses while the accumulation of reversion increased with virus passaging (Fig. 5A, B). However, the effect of TJ-32 in the type 3 vaccine viruses was lower in comparison with TJ-108 (Fig. 5B).

3) Suppressive effect of herbal constituents on reversion of polio vaccine viruses

To clarify the herbal constituents which take part in the suppressive effect, the effects of Ginseng Radix and Glycyrrhizae Radix (common herbal constituents in TJ-32 and TJ-108) on the reversion of polio vaccine viruses were analyzed by
Fig. 6 Electrophoresis of polyacrylamide gel containing (A) Alu 1, (B) ScrF 1 and (C) Mbo 1-digested PCR products of the type 1 and 3 viruses passaged with herbal medicines by non-RI MAPREC. PCR products (92, 75 bp in type 1 and 93 bp in type 3) and restriction enzyme-digested PCR products (60, 32 and 42, 33 bp in type 1 and 61, 32 bp in type 3) are shown by arrows. 1, 2 and 3 refer to the passage number. (Gi): extract of Ginseng Radix. (Gl): extract of Glycyrrhizae Radix.

The electrophoresis patterns of type 1 and 3 viruses by non-RI MAPREC are shown in Fig. 6. After amplification of cDNA by PCR, the PCR products (92, 75, 93 base pairs) and restriction enzyme-digested PCR products were separated in a polyacrylamide gel. The Alu 1-digested (60, 32 base pairs), ScrF 1-digested (42, 33 base pairs) DNA bands in type 1 viruses or Mbo 1-digested (61, 32 base pairs) DNA bands in type 3 viruses appear intensively when the revertants on 480-A, 525-C or 472-C are in abundance. In the viruses treated with TJ-108 and TJ-32, the fluorescence intensity of the digested bands became slightly weak while the digested bands appeared clearly in the control viruses passaged the third time (Fig. 6A, B, C). The suppressive effects of both medicines on reversion were observed. The results in this study support the view described in Fig. 5. On the contrary, the suppressive effects of Ginseng Radix and the non-RI-modified MAPREC method. Cultivations of the cells and viruses were performed in the presence of each herbal constituent with the highest concentrations (each 2.0 mg/mL), which did not inhibit the proliferation of the cells and viruses (data not shown).

The electrophoresis patterns of type 1 and 3 viruses by non-RI MAPREC are shown in Fig. 6. After amplification of cDNA by PCR, the PCR products (92, 75, 93 base pairs) and restriction enzyme-digested PCR products were separated in a polyacrylamide gel. The Alu 1-digested (60, 32 base pairs), ScrF 1-digested (42, 33 base pairs) DNA bands in type 1 viruses or Mbo 1-digested (61, 32 base pairs) DNA bands in type 3 viruses

Fig. 7. Fluorescence intensity of (A) Alu 1, (B) ScrF 1 and (C) Mbo 1-digested DNA bands in type 1 and 3 vaccine viruses passaged with herbal medicines by non-RI MAPREC. The digested DNA bands with high molecular weight were determined quantitatively using a VersaDoc 5000 imaging system. The experiment was performed three times; geometric means and S. D. values are described. * P < 0.05 vs. control virus.
Glycyrrhizae Radix were hardly observed, but there was a small effect in the mixture of both herbal constituents (Fig. 6A, B, C).

Furthermore, the fluorescence intensity of restriction enzyme-digested DNA bands (high-molecular-weight) in type 1 and 3 polio vaccine viruses during passaging is shown in Fig. 7. In comparison with the control viruses, it was demonstrated that TJ-108 and TJ-32 significantly suppressed the reversion on 480-A in type 1 and on 472-C in type 3 viruses passed the third time (Fig. 7A, C). In regard to reversion on 525-C in the type 1 virus, a statistically significant suppressive effect was not observed in TJ-108 while TJ-32 significantly suppressed the reversion (Fig. 7B). On the other hand, Ginseng Radix and Glycyrrhizae Radix hardly suppressed the reversion. However, the mixture of both herbal constituents significantly suppressed reversion on 472-C in the type 3 virus passaged the third time (Fig. 7C), and it was a little inhibitory on the reversion on 480-A and 525-C in the type 1 viruses though a statistical significance was not observed (Fig. 7A, B).

Discussion

In this study, accumulation of the reversion of polio vaccine viruses to the neurovirulent genotype was analyzed by the MAPREC and non-RI-modified MAPREC methods. MAPREC was developed by Chumakov et al. (1991, 1992); the non-RI method was later developed by Horie et al. (1998) to estimate the ratio of revertants in a virus population. MAPREC can be used as an effective method for a quality control test for OPV. It has been reported that the accumulation of reversion of polio vaccine viruses to the neurovirulent genotype on cultivation cells occurs easily at a virus cultivation temperature of 37°C (Chumakov et al.; 1994, Rezapkin et al.; 1994, Taffs et al.; 1995, Horie et al.; 1999, 2006), VAPP is caused frequently by the type 3 virus (Nkowane et al.; 1987) and epidemics of poliomyelitis by VDPV occur frequently due to the type 1 virus (Centers for Diseases Control and Prevention; 2006). Accordingly, analysis of the reversion of the type 1 and 3 polio vaccine viruses was performed in this study. Moreover, Caco-2 cells which were derived from the human alimentary tract were used in this study since the problems of VAPP and VDPV are thought to have been caused by reversions of the vaccine viruses in the alimentary tract.

We have reported that TJ-108 suppressed reversions of polio vaccine viruses (Horie et al.; 2007). TJ-108 is composed of a lot of herbal constituents, as shown in Table 1, and consequently it is extremely difficult to analyze which components take part in the suppressive effect. In this study, we demonstrated that TJ-32, which is composed of only four kinds of herbal constituents, also suppressed reversions of polio vaccine viruses. Furthermore, to clarify the herbal constituent which takes part in the suppressive effect, the effects of Ginseng Radix and Glycyrrhizae Radix, which are common herbal constituents in TJ-32 and TJ-108, were investigated. However, both herbal constituents hardly showed a suppressive effect on the reversion of polio vaccine viruses. It is quite likely that the effect is not simply due to individual herbal constituents. Actually, a mixture of both herbal constituents had a little suppressive effect on the reversion of polio vaccine viruses (Fig. 6, 7). A complicated interaction of some components of herbal constituents might take part in the effect. Further analyses of the herbal constituents and their components are necessary to clarify the mechanism of the suppressive effect.

Chumakov et al. (1994) reported that the reversion was promoted under the cellular activity is low, like overproliferation. It is considered that the replication efficiency of the virus decreases in such conditioning cells. It has been reported that the translation efficiency of virulent viruses is higher than that of attenuated viruses (Svitkin et al.; 1988, Pilipenko et al.; 1989); as a result, replication efficiency of virulent viruses is also higher than that of attenuated viruses. In such low activity conditioning cells, there is a fair possibility that the virulent revertants with higher replication efficiency at all become dominant easily than the attenuated viruses with low replication efficiency. However, in the high activity conditioning cells, it will be not easy for the revertants to become dominant since the attenuated viruses proliferate sufficiently. It will be important to maintain high
cellular activity to suppress the accumulation of revertants, and it should be confirmed whether TJ-32 and TJ-108 have enhanced the cellular activity to verify this hypothesis. Moreover, in this study, a pre-cultivation of the cells has been performed for five days by using the medium which contains the herbal medicines. It is necessary to analyze about the efficiency of this pre-cultivation of the cells.

The eradication of the wild type poliovirus will be achieved globally as a result of the polio eradication program in the near future. However, VDPV exists in the environment as long as OPV is in use and the viruses may cause a new epidemic of poliomyelitis in the world. New approaches for the suppression of the reversion of polio vaccine viruses to the neurovirulent genotype are urgently needed. TJ-32 and TJ-108 can be handled simply since administration is done orally and there are few side effects. It is expected that traditional herbal medicines like TJ-32 and TJ-108 will contribute to solving the problems of OPV.

Conclusion

We demonstrated that Ninjinto suppressed the reversion of polio vaccine viruses to the neurovirulent genotype, as well as Ninjin'yoeito. However, Ginseng Radix and Glycyrrhizae Radix, which are common herbal constituents in both medicines, hardly showed a suppressive effect, and a mixture of both herbal constituents had a little effect. It is quite likely that the effect is not simply due to individual herbal constituents. A complicated interaction of some components of herbal constituents might take part in the effect. Although further analyses of the herbal constituents and their components are necessary to clarify the mechanism of the suppressive effect, it is expected that traditional herbal medicines like Ninjinto and Ninjin'yoeito will contribute to solving the problems of OPV.

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References

Horie H, Miyazawa M, Ota Y et al. Analysis of the accumulation of mutants in Sabin


(Summary)

和漢薬によるポリオワクチンウイルスの毒力復帰変異の抑...

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背景 経口生ポリオワクチン（OPV）は、安全性と有効性が高いワクチンである。しかし、その一方で、極めて低い頻度ではあるが、ワクチン被接種者あるいは被接種者に接触した人、ポリオ（急性灰白髄炎）の発症例が報告されている。これは弱毒ワクチンウイルスが、人の消化管細胞で増殖する過程において、毒力復帰変異を起こすことによると考えられている。更に、その変異ウイルスが、糞便とともに環境中に排泄されることで、ポリオの新たな流行を世界各地で引き起こし、大きな問題となっている。

目的 我々は、和漢薬の一つである人参栄養エキスに、ポリオワクチンウイルスの毒力復帰変異に対する抑制効果が見られることを既に報告した。本研究では、人参栄養エキスの一種、人参栄養エキスの抑制効果が見られることを確認するために、単純にどれか1種類の生薬成分に起因するものをではなく、複数の成分による複雑な相互作用が関与している可能性が示唆された。その結果、どのような生薬成分群、どのようにメカニズムで毒力復帰変異の抑制を起こすのか、解析を行う必要が残されているが、人間栄養分を用いたスポーツのような和漢薬は、OPVが未だ問題の解決に大きく貢献することが期待される。

方法 ポリオワクチンウイルスをヒト消化管由来細胞を用いて、人参栄養エキス、人参栄養エキス、あるいは上記2種の生薬エキスをそれぞれ濃度含有的培地中で、3代鶏代培養を行った。これら経代ウイルスの毒力復帰変異について、遺伝子レベルでウイルスの毒力（神経毒力）の強さを測定できるMAPREC法、およびMAPREC法の一部を改良した非RI-MAPREC法で解析した。

結果 人参栄養エキスにも人参栄養栄養エキスに近い毒力復帰変異抑制効果が認められたが、人参と甘草の両生薬エキスについては、ほとんど抑制効果は見られなかった。しかし、両生薬エキスを混合して用いることで、若干の抑制効果が認められた。

結論 人参栄養栄養や人参栄養によるポリオワクチンウイルスの毒力復帰変異抑制効果は、単純にどれか1種類の生薬成分によって起こるものではなく、複数の成分による複雑な相互作用が関与している可能性が示唆された。どのような生薬成分群、どのようにメカニズムで毒力復帰変異の抑制を起こすのか、解析を行う必要が残されていながら、人参栄養栄養や人参栄養のような和漢薬は、OPVが未だ問題の解決に大きく貢献することが期待される。

キーワード ポリオウイルス、経口生ポリオワクチン（OPV）、毒力復帰変異、人参栄養、人蔘栄養