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The Relationship between the Cold Chain System and Vaccine Potency in Taiwan: (II) Oral Polio Vaccine

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ABSTRACT

The live attenuated oral poliovirus vaccine (OPV) has been used for the poliomyelitis eradication program in Taiwan for a long time. Since it is manufactured from a highly thermolabile vaccine strain of poliovirus, it should be stored under the recommended temperature of -20˚C or below. This study investigated and evaluated the efficiency of the cold chain system for maintaining the polio vaccine potency in Taiwan. We selected several health stations and local hospitals/pediatric offices in each County/City of Taiwan to take the OPV samples and test their potency from Nov. 1997 to Feb. 1998. Our results showed that the type-1 poliovirus of all OPV samples met the requirements for the potency test, showing titers were higher than the criteria of the WHO and the ROC (Taiwan) national standard. The type-2 and -3 polioviruses in most of the OPV samples showed titers higher than the criteria, with the exception of a few samples. We found that the degradation of type-2 poliovirus was associated with the thawed samples, which were stored at temperatures not low enough. The factor that caused the degradation of the type-3 poliovirus was unclear so far. Nevertheless, the results of this study indicated that the cold chain system used in Taiwan was generally satisfactory for the OPV storage.

Key words: **cold chain system, oral poliovirus vaccine, potency test.**

INTRODUCTION

Since humans are the only host of the poliovirus, eradication of wild-type poliovirus by vaccination is feasible[1]. The eradication of wild poliovirus from the Western Hemisphere is a testimony to the effectiveness of the oral poliovirus vaccine (OPV)[2]. Like most parts of the world[3], OPV (oral Sabin's vaccine) has been used in the poliomyelitis eradication program in Taiwan for many years. OPV was first introduced in Taiwan to prevent children from contracting poliomyelitis in 1973. Although an outbreak of poliomyelitis occurred in the Taiwan area in 1982[4], the immunization program reduced the reported incidences of poliomyelitis by over 70% from 1988 to 1993[1]. No wild-strain of poliovirus has been isolated since 1984[1]. It was reported that after a 3-

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dose inoculations of OPV, almost all the recipients become sero-positive against the type-1, -2, and -3 polioviruses[5]. Therefore, to accomplish the eradication program successfully, one of the important parts of the immunization program is to maintain the efficacy of polio vaccines.

Like other biological products, vaccines have to be of sufficient stability under the conditions of transport and storage to maintain their potency at the point of use[6]. The widely used OPV is a non-lyophilized live attenuated vaccine, and is also the most unstable vaccine in the WHO Expanded Program of Immunization (EPI)[7,8]. Several studies have reported the stability of OPV at ambient temperatures with regard to various stabilizers used in it[9,10]. OPV is known to retain its potency over a long period when stored at -20˚C or below[11]. In Taiwan, maintaining OPV at the recommended temperatures of -20˚C or below in a semi-tropical climate is one of the most important limiting factors in cold chain system. In this regard, batch certification of vaccines by national control authorities and the strict control of temperature and other factors, maintains the potency of OPV at a satisfactory level. However, the cold chain system in transportation and storage of vaccines in Taiwan is a three-level transport/storage system[12]. The common childhood vaccines are usually transported under 2~8˚C conditions from the level I area to the level II and II areas. In addition, the freezers of health stations, health rooms, and local hospitals/pediatric offices have different freezing efficiency for OPV storage. Some OPV must face an extreme variation in storage temperature and could enter a freezing-thawing cycle before use. Under these conditions, it can not be guaranteed that all polio vaccines are able to maintain effective titers to induce a satisfactory immune response. Furthermore, OPV is composed of three-serotype polioviruses, and the potency of each serotype poliovirus in a trivalent vaccine should be titrated by using mixtures of the other two type-specific antisera. However, only the total virus content (type-1 + type-2 + type-3) was quantified in vaccine samples retrieved from the field or used to monitor the cold chain[13]. Dr. Arya[14] has also reported that a simultaneous reduction in the titers of type-2 and type-3 polioviruses would not be evident during the quantification of the total viral infectivity.

Yang *et al*. have demonstrated the type-1 poliovirus potency of OPV samples retrieved from the three-level storage areas in Taiwan[15]. Their results indicated that the satisfactory percentage of the OPV samples are from 93.4% to 100% during 1992~1994. However, it was still unknown whether the vaccine potency of the type-2 and -3 polioviruses in the OPV had the same titers as that of the type-1 poliovirus. We have previously shown the relationship between the cold chain system and the measles vaccine/measles-mumps-rubella combined vaccine[12]. In this study, we attempted to find a correlation of the vaccine potency of OPV and the cold chain system in Taiwan, and identify the possible factors affecting vaccine potency. The OPV was randomly sampled from the level II and III areas in our epidemic prevention system and tested for their potency. Our results showed that the type-1 poliovirus potency of all OPV samples met its requirements, and their titers showed no significant difference between the level II and III storage areas. The OPV samples also maintained their type-2 and -3 polioviruses potency except for several dissatisfactory cases with lower titers. We found a correlation between the type-2 poliovirus degradation and the increase in the thawed vaccine samples during OPV storage. However, the cause of type-3 poliovirus degradation is still unknown. It is suggested that it could be due to the higher level of the minimal storage temperature of the OPV.

MATERIALS AND METHODS

I. *Materials*

Minimum essential medium (MEM) containing Earle's salts, L-glutamine, and sodium bicarbonate, heat-inactivated and qualified fetal bovine serum (FBS), antibiotics (100x, lyophilized) and trypsin/EDTA solution were all purchased from

Gibco BRL (Grand Island, NY). The neutralizing antisera of three-serotypes of poliovirus were kindly provided by SmithKline Beecham Pharmaceuticals (Belgium). The microtiter plates, tissue culture flasks, and other plastic accessories were from Corning/Costar (Nagog Park Action, MA).

II. *OPV Samples*

The polio vaccines were sampled from the health stations and the health rooms/local hospitals/pediatric offices in 23 Counties/Cities in the Taiwan area. The vaccine samples were coded and shipped with dry ice, and stored at -20˚C or below before testing.

III. *Cell and Cell Culture*

The Hep2 cells (ATCC CCL-23) purchased from American Tissue Culture Collection were established into the master cell bank (MCB) and working cell bank (WCB) in our laboratory. The stock Hep2 cultures were prepared from WCB. They were maintained in MEM supplemented with 10% FBS in a humidified incubator at 37˚C under 5% CO_2 condition. The confluent Hep2 cells were subcultured by 0.25% trypsin/EDTA solution and passed at 15 to 30 generations.

IV. *Potency Test*

The potency test was conducted according to the methods for potency testing of vaccine used in the WHO expanded program on immunization [13]. Serial dilutions of the samples were inoculated in rows of 10 wells of microtiter plates, together with trypsinized Hep2 cell suspension. For the assay of individual poliovirus serotype in the trivalent OPV, the highly specific antisera were used for neutralizing the polioviruses of the other two serotypes in OPV. The microtiter plates were incubated at 35˚C/5% CO_2 for 7 to 10 days. At the end of the incubation period, the numbers of the specific viral cytopathic effect (CPE) were counted and recorded. The $CCID_{50}$ (or $TCID_{50}$) per human dose was calculated using the Reed and Muench calculation method[16].

V. *Statistic Analysis*

The SPSS statistic software was used to analyze the experimental results of testing. The student's t test was used for analyzing the results of the OPV potency tests.

RESULTS AND DISCUSSION

During the period from Nov. 1997 to Feb. 1998, we randomly selected the health stations, health rooms, local hospitals, and pediatric offices from the level II and the level III storage areas in Taiwan as the sampling areas. In total, 79 samples of OPV from the above selected districts were tested. After the samples were collected, they were immediately transported to our laboratory for vaccine potency testing.

Our results showed that the average potency of the type-1, type-2 and type-3 polioviruses in the OPV samples were respectively, 6.401 ± 0.408, 5.063 ± 0.266 and 5.658 ± 0.358 Log_{10} $TCID_{50}$ per single human dose (Table 1). All samples met the criteria of the WHO and the national standards of the ROC (Taiwan) (not less than 6.0, 5.0 and 5.5 $Log_{10}TCID_{50}$/dose, respectively). In addition, the type-1 poliovirus potency of each OPV sample was higher than the criteria. The potency of the type-2 and -3 polioviruses in the OPV samples also met their requirements except for several dissatisfactory samples. The results of the type-1 poliovirus are the same as in previous studies by Yang *et al*. in 1995[15]. Therefore, we

Table 1. The average potency of three-serotype polioviruses in the OPV samples

	Serotypes		
	Type-1	Type-2	Type-3
Poliovirus Potency ($Log_{10}TCID_{50}$/dose)	6.401 ± 0.408[a]	5.063 ± 0.266	5.658 ± 0.358

[a] The average potency of three-serotype polioviruses was shown as mean ± standard deviation.

believe that most of the OPV in Taiwan has a good compliance with the cold chain system, especially the type-1 poliovirus in the OPV, which maintained satisfactory potency under the storage and transport conditions. The stabilizer, magnesium chloride ($MgCl_2$), currently used for stabilizing OPV, appears to be able to maintain the type-1 poliovirus potency.

Similar to our previous study for measles vaccine and MMR vaccine[12], we applied the student's t statistical analysis to compare the potency of the OPV samples stored in level II and III. Table 2 shows that the potency of the three-serotype polioviruses in the OPV samples from the level II areas were slightly higher than the ones from the level III areas. However, the potency differences were only from 0.024 to 0.068 $Log_{10}TCID_{50}$ per single human dose, and there was no significant difference between these two areas ($P > 0.05$). This result indicates that although the level III areas had less satisfactory storage environments owing to limited funds, facilities, manpower and other accessories, they still provided the sufficient ambient temperature control to maintain the OPV potency during transport and storage.

In this study, we also found that there were some OPV samples, which had lower titers of the type-2 and -3 polioviruses. Depending on the storage temperature records from level II/III, and the frozen or thawed vaccine conditions in sampling, the average potency of three-serotypes polioviruses in the thawed OPV samples were all lower than those in the frozen state (Table 3). The potency differences were from 0.036 to 0.281 Log_{10} $TCID_{50}$ per single human dose, and there was a significant difference in the type-2 poliovirus potency between the frozen and thawed vaccines ($P < 0.01$). We also found that a dissatisfactory proportion in the thawed OPV samples was higher than in the frozen ones, both for type-2 and -3 polioviruses (Table 4). Furthermore, the percentage of the dissatisfactory proportion of the type-2 poliovirus potency in the thawed vaccines reached 46.3%, much higher than the type-3 poliovirus. Although the WHO recommends 2-8˚C as a storage temperature for OPV[11] and reported that its potency could be maintained for years at 4˚C with the $MgCl_2$ stabilizer[17], these requirements mainly focus on the correlation of the cold chain system and the total virus content or type-1 potency[10]. That is, our results show that the aqueous form of the thawed OPV was highly related to acceleration of the type-2 poliovirus degradation. The type-2 and type-3 polioviruses are less thermo-stable than the type-1 virus[5]. It is possible

Table 2. The poliovirus potency of the OPV sampled from the level II and III storage areas

Storage Area	Poliovirus Potency ($Log_{10}TCID_{50}$/dose)		
	Type-1	Type-2	Type-3
Level II	6.405 ± 0.388^a	5.040 ± 0.255^b	5.690 ± 0.379^c
Level III	6.337 ± 0.396	5.024 ± 0.272	5.642 ± 0.351

[a,b,c] The statistical analysis was done by student's T test and there were no significant potency differences between level II and level III ($P > 0.05$).

Table 3. The poliovirus potency of the frozen and thawed OPV samples

	Poliovirus Potency ($Log_{10}TCID_{50}$/dose)		
	Type-1	Type-2	Type-3
Frozen OPV Samples	6.401 ± 0.167	5.073 ± 0.069^a	5.703 ± 0.135
Thawed OPV Samples	6.365 ± 0.162	4.792 ± 0.058	5.640 ± 0.141

[a] The type-2 poliovirus potency difference between the frozen and the thawed OPV samples was statistically significant ($P < 0.01$).

that the $MgCl_2$ stabilizer used for OPV may be effective for years to maintain only the type-1 poliovirus potency under thawed vaccine condition. The OPV stability may be improved by use of other stabilizers such as heavy water (D_2O)[18], the antiviral compound, pirodavir[19], or stricter thermo-controls in the cold chain system for maintaining the vaccine potency of the type-2 and -3 polioviruses.

Pipkin *et al*. demonstrated that the type-3 component of the live polio vaccine was the most labile[8]. However, in our testing results, the dissatisfactory proportions of the type-2 and trype-3 polioviruses in total OPV samples were 6.3 % and 3.8 %, respectively (data not shown). We showed that in Taiwan, the more unstable component of the OPV samples should be the type-2 poliovirus. We suggest that the major limiting factor for the type-2 poliovirus potency was the aqueous form of the thawed OPV due to temperature variation. Regarding the type-3 poliovirus, we found that degradation of the type-3 poliovirus seemed to be correlated with the storage temperature in each storage areas. According to the high-low temperature records from the sampling area, the lower the minimum storage temperature was, the more stable was the type-3 poliovirus (Table 5). Nevertheless, further investigation is needed to clarify this point. The cooling ability and stability of the freezer/refrigerator in the vaccine storage areas are important for vaccine potency of the type-2/-3 polioviruses in the OPV.

Table 4. The percentage of the dissatisfactory vaccines in the frozen and thawed OPV samples

	Percentage of Dissatisfactory Vaccines[a]	
	Type-2	Type-3
Frozen OPV Samples	28.6%	14.1%
Thawed OPV Samples	46.3%	26.0%

[a] The dissatisfactory percentage was calculated from the following formula: the percentage = $\frac{\text{numbers of the dissatisfactory samples}}{\text{total numbers of the frozen/thawed OPV samples}} \times 100$

CONCLUSION

In this study, we evaluated the cold chain system for storage of the oral polio vaccines by *in vitro* microtitration assay. The results indicated that the cold chain system in Taiwan provided a stable environment to maintain the type-1 poliovirus potency. In addition, there was no significant difference in potency of three serotypes between the OPV samples retrieved from the level II and III areas. We also found that there several OPV samples were dissatisfactory in their type-2 and type-3 poliovirus potency. The major contributing factor for accelerating type-2 poliovirus degradation was the aqueous form of the thawed OPV due to temperature variation. The cause of accelerating type-3 poliovirus degradation could be correlated with the higher level of minimum storage temperature. However, further investigation is necessary to clarify this point.

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Table 5. The average storage temperature in the type-3 poliovirus potency of the OPV samples

Type-3 Poliovirus Potency	Average Storage Temperature (˚C)		
	Maximal Temp[a]	Real Temp	Minimal Temp
Satisfactory OPV Samples	- 8.55	- 17.06	- 19.43[b]
Dissatisfactory OPV Samples	- 9.47	- 14.47	- 16.21

[a] The maximal, minimal and real temperatures were recorded from the high-low thermometer in the vaccine storage areas.

[b] The difference of the average minimal temperature between the satisfactory and dissatisfactory type-3 poliovirus potency of the OPV samples was statistically significant ($P < 0.05$).

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台灣地區疫苗效價與Cold Chain系統間之關係(II)口服小兒麻痺疫苗

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摘 要

活病毒口服小兒麻痺疫苗（OPV）被用於台灣地區小兒麻痺根除計畫已有相當長的歷史。由最不具熱安定性之小兒麻痺病毒製成的OPV，只有儲存在攝氏零下20度或更低的溫度中才能維持其安定性。本研究之目的爲評估台灣地區疫苗儲運cold chain系統是否具備維持小兒麻痺疫苗效價的能力。我們自民國86年9月至87年2月在台灣各縣（含直轄市）市中隨機選擇衛生所、衛生室及合約醫院進行疫苗抽樣及效價試驗。從試驗結果中發現，所有疫苗檢體之第一型病毒效價皆符合我國衛生署及世界衛生組織的相關規定，此外除了少數個案外，絕大部份的第二與第三型病毒亦可維持其效價。造成第二型病毒效價降低的原因可能與儲存時小兒麻痺疫苗是否處於溶解狀態有關，至於造成第三型病毒效價降低的原因仍不清楚，但上述第二與第三型病毒活性降低現象可能與儲存疫苗之冷凍櫃/冰箱效率不佳有關。本研究的結果顯示台灣地區所使用的cold chain系統已能提供活病毒口服小兒麻痺疫苗適當的儲存環境。

關鍵詞： cold chain系統，麻疹疫苗，麻疹，腮腺炎，德國麻疹混合疫苗，效價試驗。