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EFFICACY OF ORAL POLIOVACCINE MADE IN HUMAN DIPLOID CELLS

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The efficacy of Sabin trivalent oral poliovaccine produced in human diploid cells (HDC) was studied in 3000 children who were immunized with three doses of vaccine at 4-6 week intervals. The effectiveness of the vaccination programme was studied in a serological survey by applying the microneutralization test to blood samples collected on filter paper discs. Serum and virus mixtures were kept either at 37°C for two hours or four hours at the same temperature followed by overnight storage in a cold room. In the latter case the percentage of detectable neutralizing antibodies (1 : 10) was frequently higher. The overall seroconversion was satisfactory.

The dramatic fall in the annual inciednce of paralytic poliomyelitis in most countries of the world was mainly the result of the introduction of Sabin live oral poliovaccine (OPV) produced in primary monkey kidney (MK) cells. It was soon known that MK cells have several disadvantages: The cells frequently harbor simian and some other viruses which may be dangerous to man and , therefore, contaminated cells must be exclused in the production programme. In a recent study we have found that out of eighteen lots of MK cells, twelve presented cytopathic effect (CPE) related to some contaminant viruses, four showed unknown hemadsorbing agents, and only two lots were apparently free from adventitious viruses. Another difficulty is that monkeys are not always available and normally yield small amounts of cells. Because of these problems, a safer cell system has always been sought.

The human or primate cell lines derived from malignant or normal tissues have not been considered suitable for viral vaccine production since they normally initiate tumors in hamsters and have other characteristics of cancer cells.

The introduction of human diploid cells (HDC) by Hayflick and Moorhead (1) represented a great success in the field of production of viral vaccines. These cells are normally free from contaminants, are highly susceptible to polio and

other human viruses, and have exhibited no tumor inducing properties in small rodents or in cancer patients.

The first live polio vaccine in HDC was made with Koprowski Type 3 strain (WM-3) and was used in Yugoslavia by Ikic et al. (2) in comparison trials with the same type of vaccine made in MK cells. Between 1963 and 1966, 200,000 individuals were vaccinated in Yugoslavia with OPV produced in HDC (3,4). According to Ikic et al. (5), no difference was noticed in the efficacy and safety of the vaccines made in MK cells or in HDC. The live OPV prepared in HDC was also used in the U.S.A., Sweden (3), Switzerland (6), and in several other countries.

In Iran, Sabin trivalent OPV was first produced in HDC (7) for mass vaccination. Before general distribution and use of this vaccine was permitted, it was decided to study, in a limited field trial, the efficacy and the safety of the vaccine in susceptible children. The serological findings of this study are presented in this report.

MATERIAL AND METHODS

Administration of Vaccine and Blood Sample Collection

Children of both sexes between the ages of three months to five years, attending some health centers in Tehran (population over 6 million) and in Karaj (a town of 100,000 inhabitants near Tehran), were selected for this study.

Three doses of trivalent Sabin OPV made in HDC in our laboratory were given four to six weeks apart to the children. Each dose contained 10^6 TCD50 of Type 1, 10^5 TCD50 of Type 2 and $10^{5.5}$ of Type 3 poliovirus. The blood sample collections took place three to four months after the third vaccine administration. Finger blood sample collections from about 3000 immunized children were absorbed onto squares of filter paper discs of Whatman filter paper used for chromatography. Finger blood of another 300 children, also between the ages of three months to five years but without a previous history of vaccination, was also taken in the same way as controls. Preliminary experiments showed that 0.16 ml of blood was needed to cover the surface of each filter disc measuring 3cm. by 3cm. The age and sex of each child was recorded along with a code number for each sample. The filter - papers soaked with blood were stored at $+4°C$ before testing.

Serology

The microneutralization test was performed as described by Dömök and Magrath 1979 with the modification that Vero cells were used instead of Hep-2 cells.

The blood specimens of the immunized children were divided into two groups. The diluted sera of the first group, consisting of 850 samples, were mixed with each type of polio virus and were kept two hours at 37°C before testing (Series A). The diluted sera of the second group, which included 640 samples, were also mixed with the same polioviruses but the mixtures were kept four hours in an incubator at 37°C followed by overnight incubation in a cold room at 4-6°C (Series B). The rest of the filter discs, which were not fully covered with blood, were rejected. The titre of the challenge virus was between 30 and 60 TCD50 per well. Three stocks of virulent polio Type 1 (Mahoney), Type 2 (MEF1) and Type 3 (Saukett) were used throughout this study.

RESULTS

a) Control Children

The existing antibody level to poliovirus is reflected in Tables 1 to 4. According to the data presented in these tables, the antibody level of 41 to 43% of this group is less than 1:10 to all three types of poliovirus while only 10% show antibody level more than 1:10 to all. It is also found that 27 to 31% of the control children have antibody level less than 1:10 to two types and 17 to 20% to one type of polioviruses.

Another point of interest noted in this group of children is that, irrespective of the duration of serum-virus mixture, there is no significant difference in antibody concentrations.

b) Immunized groups

The antibody status of immunized children is given in Tables 5 to 8. The seroconversion in children under one year (Series A) against Types 1 and 3 are lower than 75% , which is normally considered to be a satisfactory response (Table 6). The better response of children under one year in Series B (Table 8) indicates the necessity of longer periods of contact between serum and virus in order to obtain a higher neutralization rate. The seroconversion of children under one year against Type 2, 78% in Series A and 82% in Series B, is quite satisfactory.

The percentage of children aged one to five years with antibody to all three types is high, reaching 95% in case of Type 2 (Tables 6 and 8) . The differences in seroconversion rates by different ages in both series A and B are not statistically significant.

The antibody pattern and the concentration of neutralizing antibody in immunized groups are presented in Tables 5 and 7. According to the data presented in these tables it is evident that when serum and virus mixtures are held four

hours at 37°C and overnight at cold room temperature the neutralizing antibody against Type 1 and 3 can be detected in a greater percentage of children. the serological response against Type 2 was high, irrespective of the duration of contact by the serum virus mixture.

DISCUSSION

The safety and the efficacy of Sabin live OPV have been the subject of a great number of studies during the last two decades. It is generally agreed that Sabin OPV, produced in primary monkey kidney cells, induces no significant untoward clinical reactions and initiates an excellent immunity. The vaccine produced in human diploid cell strains such as wi - 38 or MRC - 5 has also shown the same virtues in limited field trials.

In Iran OPV has been imported from Western countries during the last ten years. The imported vaccines were manufactured in MK cells. Since 1975 OPV made in HDC in the Razi Institute has been used in the country. Before release of large amounts of vaccine , it was decided to feed 5000 children, three months to five years old, three times with this trivalent vaccine at intervals of 4-6 weeks, in order to evaluate its safety and efficacy.

The innocuity of the vaccine was observed by teams of physicians in charge of the field trials. Seventeen million doses of OPV - HDC have since been distributed in Iran. To our knowledge, no untoward reactions have been observed. No cases of vaccine-associated paralytic poliomyelitis have so far been reported.

From the epidemiological point of view it was important to study the detectable neutralizing antibody in susceptible children before administration of vaccine as well as after use of OPV. The fact that in susceptible children only 10% have developed a neutralizing antibody to the three types of polioviruses and 27 to 31% were susceptible to two types of polioviruses, supports the urgent action of health authorities to implement a mass immunization programme. Under this programme, which was started in 1975, children aged three months to five years in cities, towns and villages with populations of over 5000 have received three doses of OPV in winter time at 4-6 weeks intervals.

It is interesting to note that when the serum of immunized children under one year was mixed with a challenge dose of polio virus Type 1 or Type 3 and mixture was tested after two hours of contact at 37°C, the percentage of children showing detectable antibody (titre of 1: 10 being evidence of immunity) was 70 and 60 for Types 1 and 3, respectively. But when the mixtures of serum and virus were held four hours at 37°C and overnight in a cold room (4-6°C) the percentage of children having detectable neutralizing antibody increased to 77 and 76, figures which represent a satisfactory response.

The better responsiveness to Type 2 poliovirus is also demonstrated in this study. This is in agreement with the findings of the British investigators (9) who observed that , of the three types of Sabin vaccine, Type 2 «takes» most readily. The percentage of immunized children, aged one to five years, showing neutralizing antibodies is mostly over 75%, regardless of the duration of serum - virus contact, and reaches 95% .

However, from the present study we can assume that when serum and polio virus Type 1 or Type 3 are in contact for longer periods of time, a higher percentage of sera show neutralizing antibody (1: 10 or more).

The fact that 15% , 13% and 18% of the children in this study lacked detectable neutralizing antibodies to poliovirus Type 1,2 or 3, respectively (Table 7), does not mean that these children are not protected. Sabin (10, 11) has shown that intestinal immunity is present in spite of negligible serum levels of antibody. According to McLeod (12) no one assumes that the absence of a virus neutralizing of 1: 10, or any measurable titre, is evidence of susceptibility. It is, of course, merely lack of evidence of susceptibility. Finally, Krugman et al. (13) have demonstrated that all sera negative by the microneutralization test show neutralizing antibody when tested by the plaque reduction method. In the light of this study, we can assume that Sabin trivalent OPV - HDC is a fully effective prophylactic which initiates immunity in nearly all susceptible children.

We thank professor Albert Sabin for his many suggestions and advices.

Table 1 - Polio Neutralizing antibodies before use of OPV

Series A

Total Children	Sex		Antibody Pattern			Age(months)						Total	%
	Male	Female	Type 1	Type 2	Type 3	3-6	%	7-12	%	13-60	%		
134	62	72	0	0	0	18	32	29	51	10	17	57	43
			+	0	0	4	40	5	50	1	10	10	7
			0	+	0	8	50	4	25	4	25	16	12
			0	0	+	5	46	2	18	4	36	11	8
			+	+	0	4	36	3	28	4	36	11	8
			+	0	+	1	20	2	40	2	40	5	4
			0	+	+	2	18	5	46	4	36	11	8
			+	+	+	4	31	2	15	7	54	13	10
						46		52		36		134	100

0 = Absence of antibody in serum dilution of 1:10

+ = Presence of antibody in serum dilution of 1:10

Table-2 Concentration of Neutralizing antibody in Children before use of OPV

Series A

Serum dilution	Antibody Pattern					
	Type 1		Type 2		Type 3	
	No of Children	%	No of Children	%	No of Children	%
< 1:10	95	72	82	62	94	70
1:10	7	5	8	6	16	12
1:20	6	4	11	8	11	8
1:40	12	9	11	8	3	2
1:80	9	6	9	7	5	4
1:160	3	2	7	5	4	3
1:320	2	2	3	2	0	0
≫ 1:640	0	0	3	2	1	1
	134	100	134	100	134	100

Table 3 - Polio Neutralizing antibodies before use of OPV
Series B

Total Children	Sex		Antiboy Pattern			Age (months)						Total	%
	Male	Female	Type 1	Type 2	Type 3	3-6	%	7-12	%	13-60	%		
103	53	50	0	0	0	23	55	16	38	3	7	42	41
			+	0	0	4	40	3	30	3	30	10	10
			0	+	0	5	46	4	36	2	18	11	10,5
			0	0	+	5	42	4	33	3	25	12	11
			+	+	0	1	20	2	40	2	40	5	5
			+	0	+	1	20	2	40	2	40	5	5
			0	+	+	2	29	2	29	3	42	7	7
			+	+	+	3	28	4	36	4	36	11	105
						44		37		22		103	100

0 = Absence of antibody in serum dilution of 1:10
+ = Presence of antibody in serum dilution of 1:10

Table-4 Concentration of Neutralizing antibody in Children before use of OPV

Series B

Serum dilution	Antibody Pattern					
	Type 1		Type 2		Type 3	
	No of Children	%	No of Children	%	No of Children	%
< 1:10	72	70	69	67	68	66
1:10	4	4	7	7	3	3
1:20	11	10	13	13	21	20
1:40	2	2	8	7	5	5
1:80	5	5	4	4	2	2
1:160	4	4	0	0	4	4
1:320	2	2	2	2	0	0
> 1:640	3	3	0	0	0	0
	103	100	103	100	103	100

Table-5 Concentration of Neutralizing antibody after use of 3doses of OPV

Series A

Serum dilution	Antibody Pattern					
	Type 1		Type 2		Type 3	
	No of Children	%	No of Children	%	No of Children	%
〈 1:10	173	20	86	10	223	26
1:10	177	21	100	12	193	23
1:20	174	21	146	18	181	21
1:40	129	16	159	19	122	14
1:80	87	10	110	13	64	8
1:160	40	5	102	12	18	2
1:320	20	2	49	6	23	3
〉1 640	31	4	81	10	23	3
Total Children	831	100	833	100	847	100
Antibody %		80		90		74

Table 6-Distribution of Neutralizing antibody in Children according to the age after use of 3 doses of O P V

Series A

Serum dilution	Antibody Pattern of age groups														
	6-12 M			13-24 M			25-36 M			37-48 M			49-60M		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
⟨1:10	83	54	109	40	14	49	25	7	23	13	3	28	12	8	14
1:10	87	43	70	31	23	37	19	10	27	22	15	28	18	9	31
1:20	53	46	65	37	28	25	31	30	38	35	26	36	18	16	16
1:40	42	48	32	17	32	31	23	28	25	30	35	22	17	16	12
1:80	24	36	19	18	18	10	19	18	13	18	22	14	8	16	8
1:160	7	33	5	11	22	5	9	18	2	12	19	6	1	10	0
1:320	4	13	4	3	7	3	3	12	8	9	12	6	2	5	2
⟩1:640	4	28	4	9	18	7	5	14	4	10	17	8	3	4	0
Total Children	304	301	308	166	162	167	134	137	140	149	149	148	79	84	83
Antibody %	70	82	60	76	92	71	82	95	83	92	98	83	85	91	84

M=Months

Table-7 Concentration of Neutralizing antibody after use of 3 doses of OPV

Series B

Serum dilution	Antibody Pattern					
	Type 1		Type 2		Type 3	
	No of Children	%	No of Children	%	No of Children	%
< 1:10	94	15	84	13	112	18
1:10	109	17	82	13	113	18
1:20	164	26	185	29	202	32
1:40	129	20	111	18	100	16
1:80	73	11	73	12	51	8
1:160	42	7	41	6	22	3
1:320	14	2	36	6	18	3
≥ 1:640	12	2	21	3	10	2
Total Children	637	100	633	100	628	100
Antibody %		85		87		82

Table 8-Distribution of Neutralizing antibody in Children according to the age after use of 3 doses of OPV
Series B

Serum dilution	Antibody Pattern of age groups														
	6-12 M			13-24 M			25-36 M			37-48 M			49-60 M		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
(1:10	55	52	58	11	14	18	8	5	8	18	9	22	2	4	6
1:10	59	45	58	21	10	18	8	11	9	17	14	24	4	2	4
1:20	65	73	78	37	35	35	21	23	34	35	45	48	6	9	7
1:40	42	43	29	21	15	18	18	14	19	42	30	27	6	9	7
1:80	19	20	16	10	10	8	16	15	6	24	24	19	4	4	2
1:160	8	7	3	2	3	5	8	9	4	20	21	9	4	1	1
1:320	2	6	7	0	10	2	3	7	2	9	13	5	0	0	2
) 1:640	0	3	1	4	7	0	2	1	3	6	8	5	0	2	1
Total Children	250	249	250	106	104	104	84	85	85	171	164	159	29	31	30
Antibody %	77	78	76	90	87	83	91	94	91	90	95	87	93	87	80

M = Months

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