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Research Article

PHASE-I, OPEN, UNICENTRIC CLINICAL TRIAL FOR EVALUATION OF SAFETY OF VARICELLA VACCINE, LIVE (I.P.) (OKA STRAIN) IN ADULTS

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ABSTRACT

The Varicella Zoster Virus (VZV) causes Chicken Pox in almost 100% children by the time they are 10-12 years old. The disease is generally mild but result in sickness lasting over 7-12 days, VZV is transmitted by air borne droplets via upper respiratory tract or by fluid from the vesicles. Varicella Vaccine, Live (I.P.) Brand name Bio PoxTM has been manufactured by Bio-Med (P) Ltd. It is a preparation of Live, attenuated OKA strain of Varicella virus. The Oka strain virus is propagated in MRC-5 human diploid cell culture containing ≥ 2000 PFU of Live, attenuated Oka strain of Varicella virus in single dose of 0.5 ml. The storage temperature recommended for Bio PoxTM is +2⁰C to +8⁰C. The vaccine batches used in clinical trial studies has been found to be safe in laboratory animals, highly stable when stored in refrigerator at +2⁰C to +8⁰C in freeze dried form. The three consecutive batches of vaccine submitted to the Central Drug Laboratory CRI Kasauli (H.P.) have been found to be of standard quality & thus merits clinical trial. The safety of Varicella Vaccine, Live (I.P.) has been evaluated in 10 healthy subjects for the period of 30 days. The vaccine was manufactured as per specifications in the Indian Pharmacopoeia monograph. The health status of volunteers were also evaluated by testing the blood & urine sample, (Dr. Lal Path Laboratory) no any noticeable abnormalities in blood counts, liver function, kidney function & routine urine test during the tenure of clinical trial was found.

Key words: Varicella Vaccine I.P., safety, Phase I Clinical trial.

INTRODUCTION

VZV is transmitted by air borne droplets via upper respiratory tract or by fluid from the vesicles. It is highly contagious with high secondary attack rates in the susceptible contacts. Increased morbidity has been reported in pregnant woman and foetus especially in first trimester.

Clinically Chicken Pox is associated with fever (100° - 103° F), malaise and generalized vesicular rashes which are concentrated on head and trunk. The rashes appear in crops over 5-6 days. The disease is of self limiting nature except in complicated case which includes bacterial super infection resulting in pneumonia, cellulites, osteomyelitis and neurological complications. Incubation period is 10-21 days. Second attack of disease is rarely reported (Weller 1965). VZV infection may be treated with oral or I/V acyclovir, famcyclovir and valacylovir (Gershon *et al.*, 2004). The affected foetus is at high risk of severe disease and consequences (Gershon *et al.*, 2008).

Pre-Clinical Study

Varicella Vaccine, Live (I.P.) is a freeze dried preparation of attenuated Oka strain of Varicella Zoster Virus obtained by the propagation of the virus in MRC-5 human diploid cell culture. The Oka strain is recommended by Indian Pharmacopoeia, European Pharmacopoeia and WHO, Technical Report Series.

The Oka strain was characterized by different tests like specific nature cytopathology sterility test, test for Mycoplasma, identity test, and test for extraneous agents in cell culture, bacterial media and animal inoculation, for mycobacterium etc. The results were satisfactory and passed the test specifications.

In the pre-clinical studies, no abnormal or adverse effects due to Varicella Vaccine, Live (I.P.) were noted in animals during the acute toxicity studies as well as long term toxicity studies. The Varicella vaccine has passed the pre-clinical studies in animals, hence it can be recommended for clinical trial in human subjects.

Stability Study

The stability study was carried out on three bulk lots of Varicella Vaccine (Live) I.P. (Bio PoxTM), under normal storage conditions (at or below -60° C) and by accelerated stability test (-20° C to -25° C).

Takahashi *et al* (1984) developed the attenuated live virus vaccine by adapting the infectious virus named "Oka" strain in guinea pig embryonic cell culture and passaging in human diploid cells. It was found suitable as vaccine for human use. The vaccine has been used in millions of children. It has been found highly efficacious in preventing the clinical disease with minimal reactogenicity in Japan, Europe, U.S.A and several countries of the world including India.

Two double blind placebo-controlled studies of Varicella vaccine have been reported one in U.S.A. and the other in Finland. Both these clinical trials represented proof of concept

of significant protection due to vaccination (Francisco *et al.*, 1996, 2000, Leroeux *et al.*, 2012). Considering the popularity of use of vaccine in India and the World at large to avoid suffering from vaccine preventable diseases, Bio-Med (P) Ltd., Ghaziabad (U.P.), in its Research & Development Labs have developed the complex methodologies involved in the manufacturing of Chicken Pox vaccine from “Oka” strains. The conditions of study and number of batches considered are satisfactory. The methods used and the results proved good stability of the product under recommended storage conditions (at or below -60°C) for at least 2 years and at accelerated condition (-20°C to -25°C) for three months. The stability of the final lot of Varicella Vaccine (Live) I.P. (Bio Pox™) under normal conditions (2-8°C) and accelerated conditions (30-35°C, 20-25°C) was studied in batches originating from different bulk lots. The methods used are similar to those used for finished product release. The test results proved good stability of the product. Test specifications for release of final lot were met after storage at recommended storage condition (2-8°C) for at least 30 months.

MATERIAL AND METHODS

Clearance and Approvals

The proposal to conduct the clinical trial was discussed independently by the Ethics committee of LLRM Medical College Meerut (U.P). No objection certificate for conducting the phase-I clinical trial, was given by the Drugs Controller (General) of India (D.C.G.I) vide letter No. F.No.12-32/BioMed/11-BD on dated September 18, 2012.

The Clinical Trial was also registered in Clinical Trial Registry of India (CTRI). The CTRI No. is CTRI/2012/11/003156 registered on 29-11-12 & acknowledgement No. is REF/2012/11/004207 & International Conference on Harmonization (ICH) – 1990 guidelines.

Vaccines

The vaccine used in the phase-I clinical trial was produced by the propagation of Oka strain of Varicella Zoster Virus in MRC-5 human diploid cell culture. The Oka strain has been recommended by India Pharmacopoeia, European Pharmacopoeia & WHO. The vaccine (BIOPOX™) contains ≥ 2000 PFU of live attenuated Varicella virus Oka strain. The manufacturing facility meets the requirement of cGMP guidelines of revised schedule M of Drugs and Cosmetic Act, Government of India.

Three consecutive Varicella vaccine batches were manufactured and all Quality control tests were conducted as per Indian Pharmacopoeia. All the reports & results have been submitted to Central Drugs Laboratory, CRI, Kasauli (H.P.).

The vaccine batches were passed by the Central Drugs Laboratory, CRI Kasauli (H.P.) certified that the vaccine batches were of standard quality & were safe for the purpose of conducting clinical trial.

Subjects

The clinical trial was unicentric open label type conducted in adults P of 18 to 55 years. The clinical trial was conducted on 10 subjects.

The 11 volunteers were screened, out of which 10 were enrolled. This number was kept small so the subjects could be directly observed by the Principal Investigators. Participated volunteers were healthy and enrolled as per inclusion & exclusion criteria of protocol.

Inclusion criteria

For the participation in clinical trial the subjects should be healthy adult of 18-55 years of Indian origin and they should remain during the study period till the end of the trial, subjects shall be aware of full information regarding the vaccine and shall give the written informed consent.

Exclusion criteria

Subjects having the history of vaccination with Chicken Pox or any adverse reaction during the previous vaccination/suffering from fever/diarrhea/any sign of acute infection/history of drug intake and allergy or hypersensitivity to any component of the vaccine/having immune deficiency or auto immune disease.

Further, the subjects participating in any other study at the same time or during the past three months or planning surgery during the study period.

Contraindications

The vaccination is contraindicated in ladies in the First trimester of pregnancy. The present study was conducted on male volunteers only.

Health status of volunteers

The Health Status of Volunteers were observed & verified by the Principal Investigator and all health parameters of subjects were filled in the Case Record Form which includes General & Systemic Examination of volunteers, Body weight, Body temperature, Heart pulse rate, Respiration rate, General appearance, Cardio vascular, Gastro intestinal, Genitourinary, Skin was observed and noted in the Case Record Form.

The volunteers who pass the Inclusion & Exclusion criteria were enrolled in the study.

Subject withdrawal criteria

Any subject may be withdrawn from the study based on decision of investigator for any inter current illness or disease. Any personal reasons of subject or family, Dropout's subjects from the study were not replaced.

Informed consent process

The subjects in the clinical trial were of age group 18 years to 55 years. They were fully informed about the purpose, risk & benefits of the clinical trial before signing the consent form. They were free to ask any doubts & query before signing the informed consent form.

Study Protocol

The clinical trial was unicentric, open label type conducted in age group 18 to 55 years adults.

Primary or Secondary end point of study

The primary or secondary end points of study were to evaluate the safety of vaccine in healthy adults. On vaccination, every subject was examined for appearance of any clinical symptom with special observation at the site of injection. The volunteers were observed for the development of fever, pain, erythema, inflammation, rashes etc. 10 healthy adults subjects were enrolled at the clinical centre for clinical trial. They were immunized by Bio Pox™ vaccine. Blood sample and urine sample was collected from the subjects on day 0 & day 30 for basic blood and urine tests conducted at Dr. Lal Pathology. The subjects were clinically monitored by the principal investigators after 30 min, 60 min, 90 min & 120 min after vaccination for any possible side effects. Subjects diaries were also provided for each subject to record any side effects during the 30 days of vaccination schedule.

Scale of Adverse Reactions

Based on the results of Varicella vaccination studies, safety assessment or scale of adverse reactions was designed for BioPox™.

Excellent :-

Local reaction (redness, induration) < 5 mm in diameter and axillary temperature < 38.3°C lasting less than 72 hours.

Good :-

Local reactions (redness, induration) 5-20 mm in diameter and axillary temperature 38.1-39°C lasting <4 days.

Fair :-

Local reaction (redness, induration) >20 mm in diameter and axillary temperature >39°C.

Bad :-

Local reaction (redness, induration) > 20 mm in diameter and axillary temperature >39°C, rashes and papule formation (≥5 numbers), vesicle formation (≥5 numbers) in 5-14 days post vaccination. The subjects were observed for development of any local or systematic adverse reactions & the result had been noted for 30, 60, 90, 120 min after vaccination.

Volunteers Records

A detailed volunteers record were maintained which contains informed consent form, CRF, a detailed demographic profile including age, sex, address, medical history & general examination. Adverse reactions were also noted in the subject diary as per protocol on every visit subsequent to vaccination.

Evaluation of safety of vaccine

The safety of the Live Varicella Vaccine had been evaluated including common side effects associated with available Live Varicella Vaccines were pain, erythema, and inflammation at the site of injection. Febrile reactions & skin rashes or papules have been reported as generalized reactions 2-14 days of inoculations.

OBSERVATIONS

Table-1

Observation & clinical parameter of 10 volunteers upto 120 minutes after vaccination

Clinical parameter	30 minutes post vaccination	60 minutes post vaccination	90 minutes post vaccination	120 minutes post vaccination	Skin Reactions observed upto 30 days	Adverse Reaction Scale Results
Pain	-ve	-ve	-ve	-ve	Absent	Excellent
Erythema	-ve	-ve	-ve	-ve	Absent	Excellent
Inflammation	-ve	-ve	-ve	-ve	Absent	Excellent
Fever	-ve	-ve	-ve	-ve	Absent	Excellent
Febrile Reaction	-ve	-ve	-ve	-ve	Absent	Excellent

Table-2

Statistical analysis of results of clinical tests done for blood & urine samples of volunteers before (0 day) and after completion of the study (30th day)					
S. No.	Variables	Pre Vaccination (Bareline) N=10	Post Vaccine (At day=30) N=10	Diff ↔ (95% conf. interval)	P-Value
1	Hb	14.4 ± 1.3	14.4 ± 1.3	0.01 ↔ (-0.27, 0.29)	0.93
2	PCV	44.0 ± 3.0	44.7 ± 2.9	-0.66 ↔ (-1.59, 0.27)	0.14
3	TLC	7.7 ± 0.9	7.3 ± 1.3	0.46 ↔ (-0.37, 1.30)	0.24

4	RBC	4.9 ± 0.5	4.9 ± 0.4	-0.07 ↔ (-0.16, 0.2)	0.11
5	MCV	91.1 ± 8.3	90.9 ± 7.4	0.16 ↔ (-1.05, 1.37)	0.77
6	MCH	29.8 ± 3.4	29.2 ± 3.3	0.51 ↔ (-0.25, 0.77)	0.00
7	MCHC	32.6 ± 1.4	32.1 ± 1.3	0.49 ↔ (-0.03, 0.94)	0.03
8	P counts	194.5 ± 44.9	198.6 ± 35.3	-4.10 ↔ (-25.71, 17.51)	0.68
9	RDW	13.6 ± 1.3	14.0 ± 1.0	-0.32 ↔ (-0.90, 0.26)	0.24
10	SGPT	73.6 ± 31.7	61.9 ± 25.7	11.7 ↔ (-3.23, 20.17)	0.01
11	GGPT	39.5 ± 23.4	33.6 ± 20.4	5.9 ↔ (-1.04, 10.76)	0.02
12	Protein	8.2 ± 0.3	7.9 ± 0.2	0.3 ↔ (0.12, 0.48)	0.00
13	Alkaline Phosphatase	106.0 ± 26.4	93.9 ± 29.9	12.1 ↔ (7.20, 17.00)	0.00
14	Creatinine	1.0 ± 0.0	0.9 ± 0.1	0.0 ↔ (-0.03, 0.09)	0.28
15	Uric acid	5.3 ± 0.9	4.9 ± 1.1	0.4 ↔ (-0.11, 0.83)	0.12
16	Sugar F	100.3 ± 21.2	96.4 ± 19.9	3.9 ↔ (-8.80, 16.60)	0.5
17	Cholesterol	175.9 ± 46.6	159.5 ± 36.8	16.4 ↔ (-1.16, 33.96)	0.06
18	Automated DLC	N,L,M,E Within Range			
18	N	4.2 ± 0.8	4.1 ± 0.9	0.1 ↔ (-0.86, 0.99)	0.87
19	L	2.5 ± 0.6	2.2 ± 0.5	0.3 ↔ (-0.07, 0.54)	0.01
20	M	0.6 ± 0.2	0.6 ± 0.2	0.1 ↔ (-0.02, 0.17)	0.12
21	E	0.4 ± 0.3	0.3 ± 0.2	0.1 ↔ (-0.10, 0.24)	0.39

RESULTS

The safety of Varicella Vaccine, Live, I.P., manufactured by Bio-Med Private Limited, Ghaziabad has been evaluated in 10 healthy subjects for the period of 30 days. After immunization with Varicella Vaccine all volunteers were observed for a period of 30 days to note any abnormal reactions occurs due to vaccination. Subject diaries were also provided to each volunteer for their home to note any adverse reaction during a period of 30 days. The volunteers were observed for 120 minutes following vaccination to observe any adverse effect both local & systemic after vaccination. None of the volunteers developed any Pain, Erythema & Inflammation at the site of vaccination (See Table No. 1). After the completion of 30 days observational period the subject diaries were taken back from the volunteers who revealed that none of the subjects noted any adverse reaction in their subject diaries.

The blood, urine samples of all volunteers were taken immediately prior to vaccination and just before completion of the study (30th day). The complete results of testing have been provided by Dr. Lal Path Lab for pre-vaccination and post vaccination blood and urine samples. No adverse opinion can be deduced from the results of the tests assignable to the vaccination. Detailed tests of hemogram, liver function, and kidney function test reveals that nothing abnormality is seen in any test performed on the subjects. Statistical analysis of the clinical test results revealed no significant changes in paired samples (Table 2). An adverse reaction scale was designed in the protocol on the basis of the previous clinical trials by other manufacturers. These studies were used to decide the adverse events in the current clinical trial.

During 30 days period the subjects had been telephonically called for confirming their Health Status by the Principal Investigator as well as by the monitor. None reported any local or systemic complication related to the vaccination. Weibel *et al* (1985) tested Live Varicella vaccine OKA strain in 1-12 years old children. They reported that the vaccine was well tolerated; however frequency of Varicella like rash was reported in 3% (4/137). The rashes were mild. The observations could be due to participation of younger children in larger numbers.

It was found completely safe and caused no local or systemic reactogenicity in any of the volunteers. No biochemical changes were observed in samples taken before & after 30 days of vaccination.

CONCLUSION

In view of the above observation, the Varicella Vaccine, Live (I.P.) Oka strain manufactured by Bio-Med (P) Ltd. as test vaccine has passed the phase I clinical trial in human volunteers. Thus phase II/III clinical trial studies can be of undertaken to evaluate safety & immunogenicity of vaccine as per approved protocol.

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REFERENCES

- Centers for Disease Control, Prevention of Varicella (2007). *MMWR, Morbidity and Mortality Weekly Report*, 56:1-55.
- Centre for Disease Control & Prevention (CDC) (2013). Updated recommendation for use of vari2Ig-United States, July 23, 2013.
- Francisco Diaz Mitoma (2000). Reactogenicity to a live attenuated Varicella vaccine in Canadian children, *Can. J. Infect. Dis*, 11, 97.
- Francois Meurice et al. (1996). Immunogenicity & safety of a Live attenuated Varicella vaccine (Oka/sb Bio) in healthy children. *J.Infect.Dis*. 174 : S324-329.
- Gershon, A.A. (2008). Varicella-zoaster virus (vzv) and its diseases WHO. Department of Immunization, vaccines and Biological, Geneva.
- Gershon, A.A., Takahashi, M and Seward, J. (2004). Varicella vaccine. In vaccines edited by Plotkin & Orenstein, Fourth Edition Elsevers Inc. U.S.A.
- Good clinical practices & schedule Y of Drugs & Cosmetic Act 1940, Government of India.
- International Conference on Harmonization (ICH) – 1990. *JAMA* 254, 2435-2439.
- Leroux - Roels I, *et al.* (2012). A phase 1/2 clinical trial evaluating safety and immunogenicity of varicella zoster glycoprotein e subunit vaccine candidate in young and older aults. *J.Infect.Dis*. 206:1280-1290.
- Takahashi, M.,Otsuka, T.,Okumo, Y,Asano, Y.Yazaki, T, Isomura, S (1984). Live vaccine used to be prevent the spread of Varicella in children in hospital. *Lancet* 2. 1288-1290.
- Weibel, RE. *et al.* (1985). Live OKA/Merck Varicella Vaccine in healthy children.
- Weller, T.H. Varicella – Herpes Zoster virus page 915-925 in *viral & Rickettsial Infections of Man*. Edited by Horsfall & Tamm, Fourth Edition J.B. Lippincott Company, Philadelphie.