RESEARCH PAPER

Immunogenicity and Safety of Live Attenuated Hepatitis A Vaccine: *A Multicentric Study*

MMA Faridi, *Nitin Shah, †TK Ghosh, $VS Sankaranarayanan, **Vidya Arankalle, Anju Aggarwal, $Malathi Sathiyasekaran, †Nisha Bhattacharya, ††T Vasanthi, $$Suparna Chatterjee, †Jaydeep Choudhury and ***Monjori Mitra

*From the Department of Pediatrics, University College of Medical Sciences, New Delhi; *Lion Tarachand Bapa Hospital and Research Centre, Mumbai; †Department of Pediatrics, Institute of Child Health, Kolkata; $Department of Pediatric Gastroenterology, Kanchi Kamakoti Childs Trust Hospital, Chennai; **National Institute of Virology, Pune; ††Department of Pediatrics, Kanchi Kamakoti Childs Trust Hospital, Chennai; $$Department of Pharmacology, IPGMER and SSKM Hospital, Kolkata; and ***Medclin Research, Kolkata, India. Correspondence to: Dr Monjori Mitra, Research Director, Medclin Research, IPHMR Building, 318/1B Prantik Pally, Kolkata 700 107, India. E mail: monjorim@vsnl.net.*

Manuscript received: June 9, 2008; initial review completed: June 27, 2008; Revision accepted: September 30, 2008.

Objective: To evaluate immunogenicity and tolerability of single dose live attenuated injectable hepatitis A vaccine in four metropolitan cities of India.

Methods: Live attenuated hepatitis A vaccine was administered to 505 children aged 18 - 60 months in four centers across India. Immunogenicity of the vaccine was assessed by estimation of anti-HAV antibody titer at 6 weeks and 6 months following administration of the vaccine. Safety evaluation of the vaccine was also done during the visits.

Results: At 6 weeks, 480 subjects (95%) came for the follow-up and 411 (81.4%) subjects reported at the end of 6 months. The geometric mean titer (GMT) of anti-HAV antibody of the subjects who did not have the seroprotective titer at the baseline were assessed at 6 weeks and 6 months which was 81.04 mIU/ml and 150.66 mIU/ml respectively. At 6 weeks, 95.1 % seroconverted and at the end of 6 months, 97.9 % had seroconverted. Both solicited and unsolicited vaccine-induced local and systemic adverse events were insignificant at all the centers, except swelling and induration in a few.

Conclusion: Live attenuated injectable hepatitis A vaccine was immunogenic and tolerable with minimal reactogenecity, in this study of single dose schedule. Safety profile was also satisfactory in the study population.

Keywords: *Hepatitis A vaccine, Immunogenicity, Live attenuated, Safety, Single dose.*

Hepatitis A virus (HAV) infection is common throughout the developing world and is frequently acquired during early childhood. Though HAV commonly affects children but adolescent and adults are also at risk in India. Countries in transition from developing to developed economies are likely witness a shift in disease prevalence from high to intermediate endemicity, and HAV is likely to become a more serious problem in these areas. The aftermath of this changing endemicity pattern will alter the incidence of the morbidity and mortality of the disease and make the adolescent and adult population more vulnerable.

In India, seroprevalence ranged from 32% to 80% in children younger than 10 years age in different demographic profile(1-3). Report of an explosive outbreak of hepatitis A involving 1180 adult cases from Kerala provides an example of

epidemic potential of HAV in India with respect to the changing epidemiology(4).

In this changing scenario, WHO has recommended large scale hepatitis A vaccination programs in areas which are prone for epidemic outbreaks. Two types of HAV vaccines are now available in the market, namely, inactivated vaccine and live attenuated vaccine. Two doses are recommended at an interval of 6–12 months in the inactivated vaccine(5), but in case of live attenuated vaccine only a single dose is recommended by the inventor and the manufacturer in the country of origin (China). WHO has encouraged studies addressing the duration of protection following a single dose of the vaccine(6).

In India, the first study on hepatitis A live attenuated vaccine was undertaken at Pune, in which the vaccine was shown to be immunogenic(7). The present multicentric study was carried out in Delhi, Mumbai, Kolkata and Chennai to measure immunogenicity, safety and geometric mean titers of the anti-HAV antibodies up to 6 months following immunization with a live attenuated hepatitis A vaccine.

METHODS

A prospective, multicentric, open labeled hospital based study was conducted at the four metropolitan cities of India, namely Delhi, Mumbai, Kolkata and Chennai from April 2007 to February 2008 amongst 505 subjects. Estimating higher prevalence of seropositivity in the older age group, the age wise sample distribution was 75% below 4 years and 25% above 4 years. The study was initiated at each center after obtaining approval of the study protocol from the ethical committee of the respective institution and the study was conducted in accordance with ICMR guidelines for biomedical research on human subjects and the Declaration of Helsinki. Subject recruitment was commenced only after explaining the matter in the informed consent form printed in local language and was signed by the parents/ guardian of the child.

After enrolling the subjects, the present and past medical history, the demographic and socio-economic profile of the study subjects were transcribed on to the case record forms. Clinical examination was conducted and blood samples were drawn at the baseline for complete blood count, serum bilirubin levels, liver enzymes and anti- HAV antibody estimation. All biochemical, hematological and serological test were conducted at SRL Ranbaxy Laboratory, Mumbai.

The freeze-dried live attenuated hepatitis A vaccine (H2 strain) developed by Zhejiang Pukang Biotechnological Company Ltd., China was used in the study. Live attenuated hepatitis A vaccine was shipped and stored at temperatures ranging from 35.6°F (2°C) to 46.4°F (8°C). The vaccine was reconstituted immediately before vaccination and injected subcutaneously in the deltoid region.

Immunogenecity of the vaccine was assessed by estimation of anti-HAV antibody titer at 6 weeks and 6 months following administration of the single dose of the vaccine. After vaccination the child was observed in the immunization clinic of respective centres for one hour to watch for immediate adverse vaccine reaction. The parents were advised to report to the hospital for any untoward incidence and to keep a record of fever, local pain, erythema and induration at the site of vaccination. Further safety evaluation was done by clinical examination and comparison of any changes from the baseline of liver enzyme and complete blood count (CBC) at 6 weeks following vaccination.

The method used for the quantitative estimation of HAV antibody in this study was Axsym® - HAVAB 2.0 Quantitative assay, based on the principle of Microparticle Enzyme Immunoassay (MEIA), on the kit Abbott Axsym. Although an immune level for anti-HAV antibodies has not been established(8), results of previous studies have suggested that values of approximately 10 mIU/ mL(9) to 20mIU/mL(10) may be indicative of immunity. *Invitro* studies using cell-culture-derived virus indicate that low levels of antibody (e.g., less than 20 mIU/mL) can be neutralizing(11). HAV vaccine responders were defined as those subjects who converted from an initially seronegative to a seropositive status using the different cut-off levels, 10 to 20mIU/ml as seroprotective. For comparison of geometric mean values and 95% Confidence Intervals (CIs), and comparisons over different

TABLE I HAV SEROPREVALENCE AT BASELINE IN CHILDREN ACROSS 18 – 60 MONTHS AGE

Anti-HAV Antibody Titer	18-24 months (*n*=75)		25-36 months (*n*=179)		37-48 months (*n*=123)		49-60 months (*n*=126)	
	No.	%	No.	%	No.	%	No.	%
0.00–0.99	40	53.33	66	36.87	43	34.95	39	30.95
1.00–4.99	15	20.00	54	30.16	40	32.52	37	29.36
5.00–9.99	2	2.66	4	2.23	1	1.62	2	1.58
10.00–14.99	1	1.33	0	0	1	0.81	0	0
15.00–9.99	1	1.33	3	1.67	0	0	0	0
>20.00	16	21.33	52	29.05	38	30.89	48	38.09

age-groups, Students' *t*-test was used for parametric data. All analyses were 2-tailed with *P*=0.05 as the cut off level for statistical significance. SPSS for Windows version 11.0 (Illinois, Chicago: SPSS Inc., 2002) software was used for statistical analysis.

RESULTS

Five hundred and five subjects in the age group of 18 to 60 months were enrolled in all the four centres irrespective of their baseline seroprevalence status. Demographic data like location, age, sex and drinking water supply, sanitary and socioeconomic conditions were recorded. Of the total 505 subjects enrolled in the study, 480(95%) subjects came for follow up at 6 weeks and 411 (81.4%) subjects reported at the end of 6 months. Serum specimen was collected during each visit to estimate anti-HAV antibodies along with complete blood counts and liver enzymes. Due to inadequate quantity of serum sample during baseline estimation, two subjects were excluded from the study subsequently.

The seroprevalence of anti-HAV antibodies before vaccination is shown in ***Table* I**. It was seen that the seroprevalence at baseline was 33% across all age groups (21% in children <2 years old, 30% in those 2 to 4 years of age, and 38% in those >4 years of age). Seroprevalence also varied in different cities and was lowest in Chennai, around 18% only.

The anti-HAV antibody geometric mean titer (GMT) was assessed at 6 weeks and 6 months for the baseline seronegative subjects only, after single dose of the live attenuated HA vaccine. ***Table* II** shows the seroconversion after 6 weeks following single dose of hepatitis A vaccine according to different seroprotective cut-off levels of antibody concentrations, which ranged from 95% to 98%.

The variation in the HAV titer values in the subsequent visits, i.e., in the first and the second visits after the baseline visit can be best studied if the interquartile range rather than simple range or variance is taken, because the effect of extreme observations and outliers can be eliminated by this process. The values found in the first and the second visits were 54.52 (first quartile=45.48, third quartile =100.00) and 101.00 (first quartile=92.25, third quartile=193.25) respectively. The subjects included were only those who were seronegative at the baseline (<20 mIU/mL) and attained an antibody concentration of >20mIU/mL after 6 weeks and 6 months. The age-wise seroconversion at 6 weeks and 6 months following single dose administration of live attenuated hepatitis A vaccine is shown in ***Table* III**. There were two non-responders in Mumbai and one in Kolkata, whose antibody concentration did not rise following vaccination.

There was no significant change in serum bilirubin and ALT values at baseline and after 6 weeks. In 1.8% cases, there was marginal rise of the enzyme which clinically was not significant as neither there was rise in the bilirubin level nor any suggested history of clinical jaundice. In the 6 weeks follow-up visit, 1.2% of the cases had raised alanine aminotransferase level but the bilirubin level was normal in all of them and none had any history of clinical hepatitis. Rest of the hematological reports was within normal limits both at the baseline and after 6 weeks of the vaccination.

TABLE II IMMUNOGENICITY OF LIVE ATTENUATED HEPATITIS A VACCINE IN CHILDREN CONSIDERING DIFFERENT SEROPROTECTIVE CUT-OFF VALUES FOR ANTI- HAV ANTIBODY TITERS

Anti-HAV antibody cut-off titers for seroprotection (mIU/mL)	Percentage Seroconversion		Post Vaccination antibody titers GMT (95 % C.I.)	
	6 weeks	6 months	6 weeks	6 months
≥H10	98.1	99.3	78.18(68.10-89.75)	144.46 (127.61-163.53)
≥H15	97.8	99.3	78.02(68.03-89.48)	143.88 (127.23-162.88)
≥H20	95.1	97.9	81.04(70.67-92.85)	150.66 (133.09-170.37)

Percentage serconversion has been calculated by the number of subjects who did not have the seroprotective titer at the baseline with the number of subjects who finally attained seroprotective antibody titer (different cut-off levels) after vaccination at 6 weeks and 6 months follow-up visit.

TABLE III AGE-WISE SEROCONVERSION* AT 6 WEEKS FOLLOWING SINGLE DOSE ADMINISTRATION OF LIVE ATTENUATED HEPATITIS A VACCINE

	At 6 weeks following vaccination					At 6 months following vaccination				
Age (months)	n1	(%)	GMT (mIU/mL)	95% CI LL	UL	n2	(%)	GMT (mIU/mL)	95% CI LL	UL
18 – 24	57	98.28	108.42	76.02	154.47	49	100.00	195.20	147.08	259.30
25 – 36	115	97.46	91.65	70.18	119.70	108	99.08	161.10	128.77	201.54
37 – 48	78	98.73	58.09	46.62	72.46	67	100.00	112.06	89.75	139.91
49 – 60	66	97.06	62.99	50.70	78.26	55	98.21	119.34	93.22	152.62
Total	316	97.89	78.02	68.03	89.48	279	99.29	143.88	127.23	162.88

n1 = number of cases at first follow up at 6 weeks. n2 = number of cases at second follow visit
**Seroconversion: subjects who did not have the seroprotective titer at baseline prior to vaccination and attained seroprotective titer after vaccination at 6 weeks and 6 months*

Both solicited and unsolicited vaccine-induced local and systemic adverse events were insignificant at all the centers, except mild swelling and slight induration in a few children. The incidence of solicited adverse events varied from 0.20 to 1.85% and the unsolicited adverse events ranged from 0.20 to 0.82%. One subject from Kolkata centre developed systemic panniculitis which was not related to the vaccine as per the opinion of the dermatologist, and it subsequently subsided and the patient came for the follow-up visit at 6 months. There were no withdrawals from any centre on account of adverse events.

DISCUSSION

The study revealed that the administration of the live attenuated hepatitis A vaccine was found to be highly immunogenic with good tolerability and minimal reactogenecity in children 18 to 60 months of age.

The seroconversion ranged from 95% to 98% at 6 weeks and 98% to 99% at 6 months considering the different cut-off levels of seroprotective antibody titers. The geometric mean titer (GMT) of anti-HAV antibody of the subjects who did not have the seroprotective titer at the baseline were assessed at 6 weeks and 6 months, which was 81.04 mIU/mL (95% CI–70.67-92.85) and 150.66 mIU/mL (95% CI–127.23–162.88), respectively following the vaccination when >20mIU/mL was considered as the seroprotective titer.

The variation in the HAV titers in the interquartile analysis revealed that the variation increased as the time gap increased. The increasing

WHAT IS ALREADY KNOWN?

- Single dose injectable live attenuated hepatitis A vaccine is immunogenic and safe in children 18-60 months in only one center in India.

WHAT THIS STUDY ADDS?

- Single dose injectable live attenuated hepatitis A vaccine is immunogenic and safe in children 18 – 60 months across different geographical areas in India.

anti-HAV antibody titers at 6 months following single dose of the vaccine might be due to the fact that this vaccine contained whole structure of the attenuated H2 strain virus particle that induced immune responses similar to the natural infection in humans. Besides this, the increase in seroconversion could be due to natural infection also. It has been reported that this vaccine can induce not only neutralizing antibodies but also the cell-mediated immune responses. As a result, the protection based on cellular immunity could persist even after anti-HAV antibodies become undetectable. When vaccinees are re-exposed to HAV, an anamnestic response may well prevent overt disease(12).

While analysing the age-wise seroconversion at 6 weeks and 6 months following single dose administration of the vaccine, the GMT at 6 weeks and 6 months were maximum in the age group 18-24 months. Thus the vaccine is highly immunogenic at an early age. In other groups, most of the subjects achieved seroprotective level at 6 months following single dose of the vaccine. More than 95% children who did not report at 6 months had seroconverted at 6 weeks. The observations show that inclusion of this group would not have changed the data significantly with the inclusion in the final analysis.

In this study, 3 subjects were nonresponders, which may be due to a lowered immunity in general or because the host's immune system did not have a B-cell capable of generating antibodies against that antigen. In a multivariate analysis, it has been seen that the CD4 count at the time of vaccination was associated with the absence in the response to hepatitis A vaccine (P<0.0001)(13). In our study, these subjects could not be followed up for the CD4 count.

We did not find serious vaccine related adverse reaction in any subject over a period of 6 months after administration of the vaccine. Excepting in few cases, subjects developed mild swelling, pain and erythema. None of the child developed clinical or biochemical hepatitis following vaccination. Although we observed the safety profile in the subjects, this study was not adequately powered statistically to comment on the safety of the vaccine as the primary outcome.

The only Indian study(7) on this vaccine has shown its high immunogenicity and excellent safety profile that is further strengthened by the present study. Studies from China also showed high immunogenicity of the vaccine and the long term studies showed persistence of protective antibody level up to 15 years following a single dose of live attenuated HA vaccine. A comparative study with single and booster dose did not reveal any significant advantage in the long term protective value with the addition of booster(12,14-16). Further studies to evaluate the long term persistence of anti-HAV antibody after the single dose of live attenuated hepatitis A vaccine in children of India, are needed.

It can be concluded that this live attenuated hepatitis A vaccine is tolerable with minimal reactogenecity and it is immunogenic in children 18 months to 5 years of age with the single dose.

ACKNOWLEDGMENT

Acknowledgment is made to Dr A Parthasarathy (former IAPCOI Chairperson), Dr Apurba Ghosh (Director, ICH Kolkata), Dr MKC Nair (Past IAP President) for their advise in the study project. Professor M Pal and Dr P Bharati of Indian Statistical Institute, Kolkata for their assistance in carrying out the statistical analysis of the study.

Contributions: SC, MMA, NS, TKG, VSS and MM were responsible for designing the study. MM was primarily responsible for day to day conduct of the study. VA, JC, MMA, TKG, AA, SC, NGB and MM drafted the paper. MM will act as the guarantor for the manuscript.

Funding: Study was conducted with funding from Wockhardt India Ltd., Mumbai.

Competing interests: None stated.

REFERENCES

1. Jindal M, Rana SS, Gupta RK, Das K, Kar P. Serological study of hepatitis A virus infection amongst the students of a medical college in Delhi and evaluation of the need of vaccination. Indian J Med Res 2002; 115: 1-4.
2. Joshi N, Yr NK, Kumar A. Age related seroprevalence of antibodies to hepatitis A virus in Hyderabad, India. Trop Gastroenterol 2000; 1: 63-65.
3. Arankalle VA, Tsarev SA, Chadha MS, Alling DW, Emerson SU, Banerjee K, *et al*. Age-specific prevalence of antibodies to hepatitis A and E viruses in Pune, India, 1982 and 1992. J Infect Dis 1995; 171: 447-450.
4. Arankalle VA, Chadha MS, Chitambar SD, Walimbe AM, Chobe LP, Gandhe SS. Changing epidemiology of hepatitis A and hepatitis E in urban and rural India (1982-1998). J Viral Hepat 2001; 8: 293-303.
5. Indian Academy of Pediatrics Committee on Immunization. Hepatitis A vaccine. In: Shah RC, Shah NK, Kukreja S, eds. Guidebook on Immunization. Mumbai: Indian Academy of Pediatrics 2006: 32-33,79.
6. WHO. Hepatitis A vaccines, Position paper. Wkly Epidemiol Rec 2000; 75:38-42.
7. Bhave S, Bavdekar A, Madan Z, Jha R, Bhure S, Chaudhari J, *et al*. Evaluation of immunogenicity and tolerability of a live attenuated hepatitis a vaccine in Indian children. Indian Pediatr 2006; 43: 983-987.
8. Lemon SM. Type A viral hepatitis: Epidemiology, diagnosis and prevention. Clin Chem 1997; 43:1494-1499.
9. Ambrosch F, Widermann G, Andre FE. Comparison of HAV antibodies induced by vaccination, passive immunization and natural infection. In: Hollinger FB, Lemon SM, Margolis HS, eds. Viral Hepatitis and Other Liver Disease. Baltimore: Williams and Wilkins; 1991. p. 98-100.
10. Berger R, Just M, Beat A. Time course of hepatitis A/antibody production after active, passive and active/passive immunization: the results are highly dependent on the antibody test system used. J Vir Meth 1999; 13: 287-298.
11. Nolan T, Bernstein H, Blatter MM., Bromberg K, Gueria F, Kennedy W, *et al*. Immunogenicity and safety of an inactivated hepatitis A vaccine administered concomitantly with diphtheria-tetanus-acellular pertussis and haemophilus influenzae type B vaccines to children less than 2 years of age. Pediatrics 2006;118: e602- 609.
12. Wang XY, Xu ZY, Ma JC, Von Seidlein L, Zhang Y, Hao ZY, *et al*. Long-term immunogenecity after single and booster dose of a live attenuated hepatitis A: Results from 8-year follow-up. Vaccine 2007; 25 : 446-449.
13. Rimland D, Guest JL. Response to hepatitis A vaccine in HIV patients in the HAART era. AIDS 2005; 19: 1702-1704.
14. Zhuang F, Qian W, Mao ZA, Gong YP, Jiang Q, Jiang LM, *et al*. Persistent efficacy of live attenuated hepatitis A vaccine (H2 strain) after a mass vaccination program. Chin Med J 2005; 118: 1851-1856.
15. Xu Z, Wang X, Li R, Meng Z, Zhang Y,Gong J, *et al*. Immunogenecity and efficacy of two live attenuated hepatitis vaccines (H2 strain and LA-1strains) Zhonghua Yi Xue Za Zhi 2002; 82: 678-681.
16. Mao JS, Chai SA, Xic RY, Chen NL, Jiang Q, Zhu XZ, *et al*. Further evaluation of the safety and protective efficacy of live attenuated hepatitis A vaccine (H2-strain) in humans. Vaccine 1997; 15: 944-947.