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Original Article

EFFECT OF VIBROMIXING ON HEPATITIS B VACCINE POTENCY

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ABSTRACT

Objective: The aim of this study is to evaluate effect of vibro mixing on potency of Hepatitis B vaccine.

Methods: Effect of vibro mixing was studied by preparing Hepatitis B vaccine formulation by using vibro mixer and comparing with formulation made using magnetic stirrer. In-vitro and in-vivo potencies were analysed for both the formulations to evaluate the vibro mixer effect. Option of use of formulation excipient to protect from shear stress during vibro mixing was explored. Nonionic block co polymer Polaxamer was selected as protective agent based on its protein stabilizing nature and suitability as pharmaceutical excipient with no toxicity at low concentrations. Protection efficiency of non ionic block copolymer from shear stress of vibro mixing in hepatitis B vaccine blending was studied by analyzing in-vitro and in-vivo potency of formulations with and without nonionic block copolymer.

Results: Hepatitis B surface antigen found losing both in-vitro and in-vivo potency with the use of vibro mixer for blending with aluminum hydroxide adjuvant. In comparison with formulation prepared with magnetic stirrer about 40% loss of potency was observed. No loss of potency was observed with inclusion of polaxamer in to formulation.

Conclusion: Vibro mixing is causing loss in potency of hepatitis B vaccine and Polaxamer 188 block copolymer at low concentrations protected Hepatitis B surface antigen from vibro mixing stress.

Keywords: Vaccine blending, Vibro mixing, Shear stress, Potency, Polaxamer.

INTRODUCTION

Vaccination is the primary means to prevent the Hepatitis B disease. Recombinant hepatitis B vaccine was introduced in 1986. The active substance in recombinant hepatitis B vaccine is hepatitis B surface antigen (HBsAg) that has been produced in yeast or mammalian cells into which the Hepatitis B surface antigen gene has been inserted using plasmids. Following thorough purification from host-cell components, Hepatitis B surface antigen is adsorbed to an adjuvant.[1]

The recombinant HBsAg polypeptide has a molecular weight of 24 kDa, and self-assembles readily in solution to form liposome-like 22 nm particles presenting antigenic HBsAg epitopes on their surface. The commercially available Hepatitis B vaccines are formulated by adsorbing hepatitis B surface antigen (HBsAg) onto aluminum hydroxide (commonly known as alum) as a liquid suspension. The adsorption of HBsAg by aluminum hydroxide adjuvant was shown to exhibit a high affinity adsorption isotherm. However, in spite of the favorable electrostatic properties, the adsorption of HBsAg by aluminum hydroxide adjuvant was shown to be predominantly due to ligand exchange between the phospholipids of HBsAg and surface hydroxyls of aluminum hydroxide adjuvant. [2]

Increasing batch size of vaccine blending helps in reducing number of batches which ultimately save production time and cost. This also helps in reducing number of animals required for release of vaccines per lot and helps in maintaining the price line for vaccines at affordable level. To increase blending size large vessels with efficient mixing option should be available. Vibro mixers gives efficient mixing at high volumes.

In this study we examined non ionic block copolymers polaxamer 188 (also called as lutrol or Pluronic (trade name of BASF)) for shear stress protection activity on aluminum adjuvanted hepatitis B vaccine. Polaxamers are non toxic synthetic copolymers of ethylene oxide and propylene oxide. Polaxamers are available in pharmaceutical grade and commercially available at low cost. polaxamers are readily soluble in water. lutrol F 68 has been used in liquid formulation for parenteral use.[3].

Vigorous agitation of aqueous solutions of interleukin 2 and Urease at 4°C resulted in a greater than 50% loss in the biological activity at 12 and 24 hours for both interleukin 2 and urease. Similar aqueous solutions which were maintained at 4 degree C and contained either urease or interleukin 2 and polaxamer 407 at a concentration of 10%w/v and 0.5%w/v, respectively lost no significant biological activity when subjected to vigorous agitation for 96 hours. In earlier time points urease showed increased biological activity [4].

At suitable concentrations in poloxamer 407, proteins form homogeneous suspensions with full retention of native secondary structure. Pluronic block polymers were found exciting opportunities for the development of novel gene therapies and vaccination strategies [5]. Proteins are compatible with aqueous gels such as those formed by poloxamers.[6]

Pluronic F127 is a nonionic, hydrophilic polyoxyethylene–polyoxypropylene (POE–POP) block copolymer previously used for its surfactant and protein stabilizing properties [7]

Aggregation is ameliorated by the inclusion of surfactants in biotherapeutic formulations, typically non-ionic polymeric ether surfactants. The most commonly used are polysorbates. triton, poloxamers, and polyoxyethylene alkyl ether. Polysorbates contain ether linkages and unsaturated alkyl chains that have been shown to auto-oxidize in aqueous solution to protein-damaging peroxides and reactive aldehydes including formaldehyde and acetaldehyde [8]. Polysorbate 20 and 80 found to be less effective in stabilization of protein exposed to stirring[9].

MATERIALS AND METHODS

Material

Hepatitis B surface antigen was from Biological E Ltd. The HBsAg was made in yeast *Pichia Pastoris*. Nonionic block copolymer polaxamer 188 was purchased from HiMedia Laboratories Pvt. Ltd. Aluminum hydroxide adjuvant was obtained from Brentag Biosector. AxSYM HBsAg V2 kit is from Abbott laboratories. All the chemicals used for testing were of analytical grade and purchased from commercial suppliers.

Methods

Stock solution of polaxamer 188 at 10% W/V concentration prepared in cold water for injection and kept at 2-8°C overnight for complete dissolution. Polaxamer stock solution was sterilized by filtration. Phosphate buffer pH 7.4 prepared by dissolving dibasic sodium phosphate anhydrous 1.12g and monobasic sodium phosphate monohydrate 1.1 g in liter of water for injection.

Formulation

Different formulations having polaxamer 188 concentrations of 0.1, 0.5 and 1% were prepared . Two control formulations without polaxamer 188, with and without use of vibro mixer (with use of magnetic stirrer) were prepared.

Formulation1: 20µg/mL of hepatitis B surface antigen, 0.5 mg/mL Aluminum hydroxide, polaxamer 188 0.1% W/V and phosphate buffer sufficient to make up the volume . Mixing at 50Hz for 2 hours using vibro mixer.

Formulation2: 20µg/mL of hepatitis B surface antigen, 0.5 mg/mL Aluminum hydroxide, polaxamer 188 0.5% W/V and phosphate buffer sufficient to make up the volume. Mixing at 50Hz for 2 hours using vibro mixer.

Formulation3: 20µg/mL of hepatitis B surface antigen, 0.5 mg/mL Aluminum hydroxide, polaxamer 188 1% W/V and phosphate buffer sufficient to make up the volume. Mixing at 50Hz for 2 hours using vibro mixer.

Control formulation 1: 20µg/mL of hepatitis B surface antigen, 0.5 mg/mL Aluminum hydroxide and phosphate buffer sufficient to make up the volume. Mixing at 50Hz for 2 hours using vibro mixer.

Control formulation 2: 20µg/mL of hepatitis B surface antigen, 0.5 mg/mL Aluminum hydroxide and phosphate buffer sufficient to make up the volume. Mixing at 100 to 150 rpm for 2 hours using magnetic stirrer.

In-vitro relative potency (IVRP)

In-vitro potency of the formulations was estimated by using Abbott AxSYM HBsAg V2 kit on AxSYM automated enzyme immunoassay system. Four different dilutions with interval of 2 fold (1000, 2000, 4000 and 8000) were prepared in phosphate buffer with bovine serum albumin. each dilution was prepared in quadruplicate. 150 µl of the diluted sample or standard, anti-HBs MAb-coated micro particles and biotinylated anti-HBs polyclonal antibody were mixed together and incubated in a reaction vessel. This reaction mixture was later dispensed onto a matrix cell. The anti-biotin-alkaline phosphatase conjugate was then dispensed onto the matrix cell, followed by buffer washes. The alkaline phosphatase activity was determined by the addition of a substrate, 4-methylumbelliferyl phosphate, which was converted to methyl umbelliferone, and the fluorescent signal was measured by the AxSYM instrument. The S/N values were calculated using an automated analyzer against the stored AxSYM HBsAg index calibration curve. [10]. Relative potency was calculated using parallel line statistical program F.R.Marsman, RIVM, Bilthoven, The Netherlands (version: 2000-1) using S/N values obtained for different dilutions of sample and standard vaccines. In-vitro potency was tested two times on each formulation.

In-vivo potency

Potency in-vivo was carried out by comparing its capacity to induce specific antibodies against hepatitis B surface antigen (HBsAg) in mice with the same capacity of a reference preparation. [11] Sera collected after 28 days from BALB /c mice immunized intra-peritoneally with 3 different dilutions is titrated by ELISA. For each dilution of standard and sample a group of 20 mice were used. Cut off value to determine sero conversion is calculated from optical densities of sera from placebo immunized mice. Any response in vaccinated animals that exceeds this level was considered as sero conversion. Probit transformation of the percentage of animals showing sero conversion in each group was done and the data was analysed according to a parallel-line log dose-response model using probit program of F.R. Marsman, RIVM, Bilthoven, The Netherlands (version 2000-1).. Potency of the test preparation was calculated relative to the reference preparation. Animal usage for the in vivo experiments were approved by institutional ethics committee of Biological E Ltd.

RESULTS

In-vitro relative potency of the formulations prepared with use of vibro mixer, magnetic stirrer and different concentrations of protective agent analysed using AxSYM system. Each formulation was analysed twice. The results of the in-vitro potency were tabulated in the table 1. Difference in in-vitro potency compared to formulation made with magnetic stirrer (control formulation 2) was calculated and the obtained difference was tabulated in table 1. Average of two test was used for calculating % difference.

In-vivo potency of the formulations prepared with use of vibro mixer, magnetic stirrer and different concentrations of protective agent analysed by mice potency assay. The obtained results of the in-vivo potency with 95% confidence limits were tabulated in the table 2. Difference in in-vivo potency compared to formulation made with magnetic stirrer (control formulation 2) was calculated and the obtained difference was tabulated in table 2. Sero conversion (ie. Number of mice sera showed optical density values higher than cut off value out of 20 immunized mice in ELISA) number for different dilutions of each formulation was graphically represented in fig2.

Table 1: In-vitro relative potency results of different formulations

Formulation	Relative potency		Average of 2 tests	%difference from control formulation 2
	Test 1	Test 2		
Formulation 1	1.2	1.1	1.15	-4
Formulation 2	1.3	1.3	1.3	8
Formulation 3	1.1	1.2	1.15	-4
Control formulation 1	0.7	0.8	0.75	-38
Control formulation 2	1.2	1.2	1.2	not applicable

Table 2: In-vivo potency results of different formulations

Formulation	Potency (Confidence limits)	% difference from Control formulation 2
Formulation 1	1.73 (1.06-2.86)	63
Formulation 2	1.67(1.04-2.71)	55
Formulation 3	1.35(0.80-2.28)	30
Control formulation 1	0.61(0.37-1.01)	-42
Control formulation 2	1.07(0.65-1.75)	Not applicable

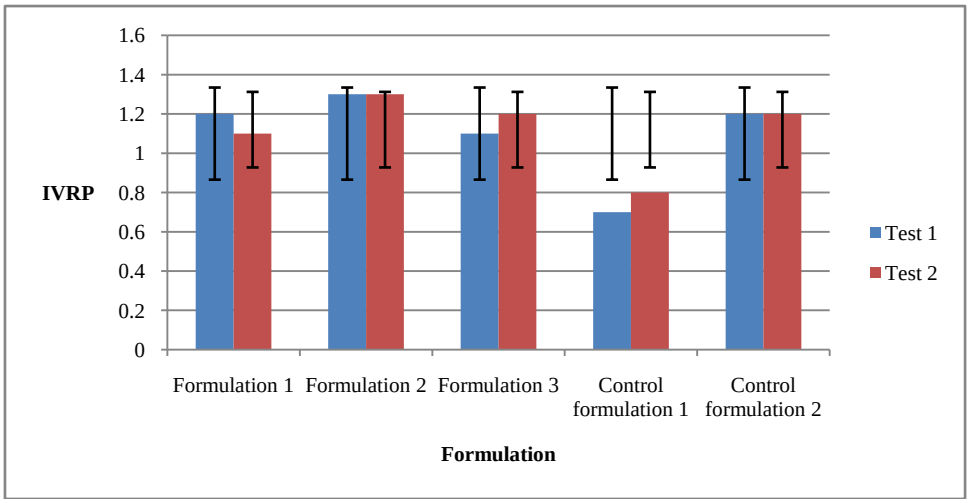

Fig. 1: Graphical representation of In-vitro potency results with standard deviation error bars

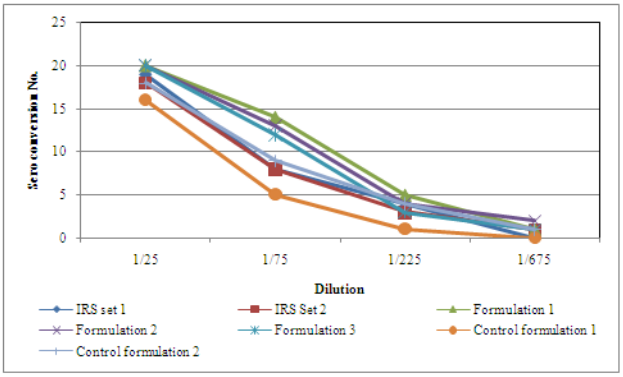

Fig. 2: Graphical representation of sero conversion in In-vivo potency assay

DISCUSSION

Formulations having similar concentrations of hepatitis B surface antigen and aluminum hydroxide prepared with use of vibro mixer and magnetic stirrer for mixing (control formulation 1 and 2). Lower in-vitro and in-vivo potency values observed for the formulation made with vibro mixer compared to formulation made with magnetic stirrer. In some cases antigen may lose its reactivity with antibody in-vitro, but can be immunogenic in-vivo. But in case of formulation made with vibro mixer loss in both in-vitro and in-vivo potency was similar. A loss of 38% in in-vitro potency and 40% in in-vivo potency was observed in formulation made with use of vibro mixer when compared to formulation made of magnetic stirrer. Hepatitis B vaccine formulations containing 3 different concentrations of polaxamer were prepared with use of vibro mixer for mixing (Formulation 1, 2 and 3). All these three formulations tested for in-vitro potency and in-vivo potency and no loss of in-vitro and in-vivo potency observed in formulations prepared with use of polaxamer as formulation excipient. Standard deviation error bars in fig.1 indicates that in-vitro potency values for the formulations prepared with polaxamer and vibro mixer were similar to in-vitro potency values obtained for formulation prepared with magnetic stirrer. Sero conversion in different dilutions for each formulation shown in Fig.2 explains less sero conversion in formulation with no polaxamer and higher sero conversion in formulations with polaxamer. Increase in in-vivo potency was observed with formulations prepared with polaxamer 188. This increase of in-vivo potency may be due to additional adjuvant effect exerted by polaxamer 188.

CONCLUSION

Investigation of effect of vibro mixing on Hepatitis B vaccine potency was carried out by analysing in-vitro and in-vivo potency of formulations made with use of vibro mixer and comparison with formulations made with use of magnetic stirrer. Investigation revealed that use of Vibro mixer in formulation of hepatitis B vaccine with aluminum adjuvant alone is not feasible and loss of both in-vitro and in-vivo potency was observed. Nonionic block copolymer polaxamer 188 found suitable as protective agent against stress induced by Vibro mixer during formulation of Hepatitis B vaccine. Presence of Nonionic block copolymer also helped in improvement of immunogenicity in animals. Polaxamer 188 at concentrations as low as 0.1% was able to produce protective activity. We can conclude that vibro mixing has deleterious effect on the potency of the hepatitis B vaccine made of aluminum adjuvant alone and nonionic block copolymer can be included in formulation to protect from vibro mixing stress.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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