Evaluation of Immunogenicity and Safety of Polymer Based Nasal Vaccine

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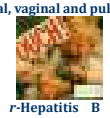
OBJECTIVES

1. To study the effect of particle size and surface charge on systemic and mucosal immune response.
2. To check the ability of chitosan derivatives as mucoadhessive polymer.
3. To investigate the stability of recombinant HBsAg within coated and uncoated PLGA Nanoparticles.
4. To develop a needle-free vaccination i.e., Mucosal administration (Nasal route).
5. To evaluate Systemic and Mucosal immune responses.

MUCOSAL ROUTES

Nasal vaccination at one site induces immunity at several distant mucosal sites (oral, rectal, vaginal and pulmonary).



Protein: *r*-Hepatitis B surface antigen (HBsAg)

Polymer: PLGA, Chitosan and trimethyl Chitosan (TMC)

Delivery Systems: PLGA / Modified PLGA Nanoparticles

PLAN OF WORK

- Preparation of PLGA Nanoparticles containing stabilized HBsAg
- Preparation of chitosan and tri-methyl chitosan (TMC) coated PLGA Nanoparticles containing HBsAg
- Characterization of Nanoparticles
- Estimation of HBsAg content in PLGA Nanoparticles/Modified PLGA Nanoparticles
- *In vitro* release studies
- Determination of structural integrity of antigen
- Measurement of ciliary beat frequency
- Fluorescence microscopy
- Particle Uptake studies
- *In vitro and In vivo* studies

PRINCIPLE INVOLVED IN THE MUCOSAL IMMUNIZATION

Nasal Vaccine Delivery:

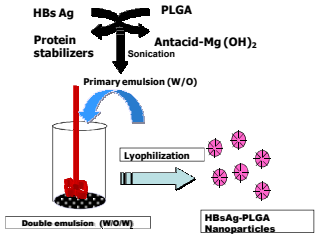
(1)Mucoadhesion;

(2)Antigen uptake by M-cell transport;

(3)Delivery to and subsequent activation/maturation of DC;

(4)Induction of B-cell and T-cell responses.

PREPARATION of HBsAg LOADED PLGA NANOPARTICLES



CHARACTERIZATION OF NANOPARTICLES

Estimation:- HBsAg by micro BCA method
BCA protein estimation kit (KT-31) (Genei., Bangalore)
Size:- Zetasizer Nano ZS(Malvern apparatus, UK)

HBsAg loaded Nanoparticle type	Size in nm n=3	PDI*	Zeta potential (mV) n=3	Entrapment efficiency (%)
PLGA-TMC-NP (85/15)	729±13.4	0.297±0.051	28±0.70	82±5.8
PLGA-TMC-NP (50/50)	445±14.6	0.236±0.032	31±0.10	79.3±5.3
TMC-NP	274±12.4	0.198±0.018	16.5±0.40	61.3±3.8
PLGA-NP	304±16.3	0.153±0.033	-44.3±0.60	33±4.2

*PDI = Polydispersity Index

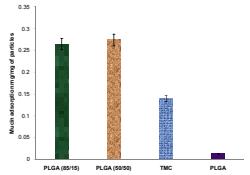
CONFIRMATION OF STRUCTURAL INTEGRITY BY SDS-PAGE

Lane 1: Marker Proteins
Lane 2: Native HBsAg (24kDa)
Lane 3: HBsAg from PLGA -TMC NP (85/15)
Lane 4: HBsAg from PLGA-TMC (50/50) NP
Lane 5: HBsAg from TMC NP
Lane 6: HBsAg from PLGA NP

SDS-PAGE analysis showing stability encapsulated antigen isolated from coated and uncoated Nanoparticles

ADSORPTION OF MUCIN ON NANOPARTICLES

- Equal vol. of nanoparticle & aqueous Sol. of mucin shaken at room temperature for 60 min.
- Centrifuged and supernatant was used to determined free mucin content
- Colorimetric assay for glycoprotein (periodic acid/schiff staining)

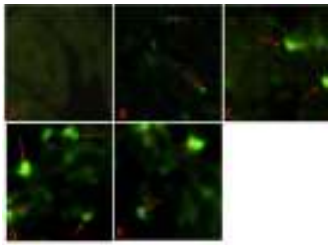


In-vitro mucin adhesion Test: Graph showing mucin adhesion ability of coated and uncoated nanoparticles

FLUORESCENCE MICROSCOPY

Fluorescence marker: FITC-BSA

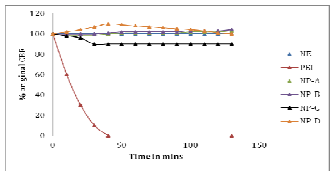
Nasal mucosa was observed under fluorescence microscopy (Nikon eclipse, E-600)



Fluorescence microscopy images showing the uptake of FITC-BSA loaded nanoparticles in NALT.
(a) Soluble FITC-BSA ; (b) PLGA NP; (c) TMC NP; (d) PLGA-TMC NP(85/15) ; (e) PLGAQ-TMC NP (50/50)

EVALUATION OF TOXICITY BY CILIARY BEAT FREQUENCY

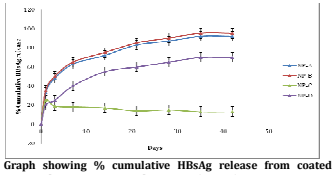
- Human nasal epithelial cells were isolated from nasal biopsies



Ciliary beat frequency after exposure to 0.5 mg/ml HBsAg-loaded nanoparticles as a measure for nasal cilia toxicity. Nasal epithelium was exposed for 45 min to formulations, after which the epithelium was washed and the CBF allowed to recover for 90 min. (n=3)

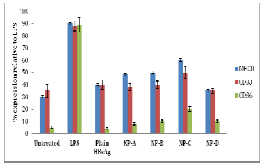
IN VITRO RELEASE STUDIES

- PBS (pH7.4, 0.1M) at 37°C
- Time points- 1,3,7,14,21,28,35 and 42 days
- Centrifuged and antigen was estimated by micro BCA method



Graph showing % cumulative HBsAg release from coated and uncoated PLGA nanoparticles in PBS (pH 7.4).

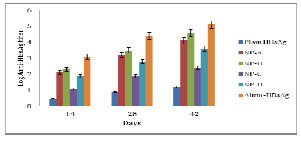
PARTICLE UPTAKE STUDIES BY DENDRITIC CELLS STUDIES



Interaction of HBsAg loaded nanoparticles with DC:Association of particles with human DC quantified using flow cytometric analysis. Bars represent mean±SD of 6 different monocytes donors.

SYSTEMIC IMMUNE RESPONSE (IgG LEVELS)

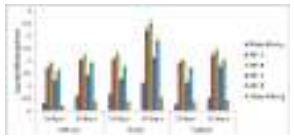
The concentration of anti-HBsAg antibody-by solid phase ELISA



Graph showing anti-HBsAg IgG titer in serum in different group immunized with native HBsAg, alum-HBsAg and developed formulations. Values are expressed as Mean±SD (n=6). Each data represents mean±S.D. (n=6).

MUCOSAL IMMUNE RESPONSE (sIgA LEVELS)

The concentration of anti-HBsAg antibody-by solid phase ELISA



Graph showing anti-HBsAg sIgA titer in various mucosal secretions of different mice groups immunized with native HBsAg, alum-HBsAg and developed formulations. Values are expressed as Mean±SD (n=6). Each data represents mean±S.D. (n=6).

CONCLUSION

Particle size and surface charge have major impact on mucosal immunity
The extent of the absorption of the antigen encapsulated into the particles was dependent on the size of the particles, being more important for the Nanoparticles than for the Microparticles.

❖Choice for the practical development of Particulate vaccine for mucosal immunization.