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Mannosylated niosomes as carrier adjuvant system for topical immunization

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Abstract

The aim of this study was to develop mannosylated niosomes as a topical vaccine delivery carrier and adjuvant for the induction of both humoral and cellular immunity. Bovine serum albumin (BSA)-loaded niosomes composed of sorbitan monostearate/sorbitan trioleate (Span 60/Span 85), cholesterol and stearylamine as constitutive lipids were prepared by the reverse-phase evaporation method. The niosomes were coated with a modified polysaccharide O-palmitoyl mannan (OPM) to target them to Langerhan's cells, the major antigen presenting cells found in abundance beneath the stratum corneum. Prepared niosomes were characterized in-vitro for their size, shape, entrapment efficiency and ligand binding specificity. The immune stimulating activity was studied by measuring serum IgG titre and its subclasses (IgG2a/IgG1 ratio) following topical application of various niosomal formulations in albino rats. The results were compared with alum-adsorbed BSA following topical application and intramuscular injection. It was observed that niosomal formulations elicited a significantly higher serum IgG titre upon topical application as compared with topically applied alum adsorbed BSA ($P < 0.05$). The serum IgG levels were significantly higher for the mannosylated niosomes as compared with plain uncoated niosomes ($P < 0.05$). All formulations displayed a combined serum IgG2a/IgG1 response, which suggested that the formulations were capable of eliciting both humoral and cellular responses. The study signified the potential of OPM-coated niosomes as a topical vaccine delivery carrier and adjuvant. The proposed system would be simple, stable, and cost effective and might be clinically acceptable.

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Introduction

Traditional parenteral methods of immunization are expensive due to the need of a sterile manufacturing process and for qualified medical personnel for vaccine administration. There is also the risk of needle borne infections (e.g. HIV or hepatitis) due to the use of contaminated needles (Kane et al 1999; Simonsen et al 1999; Jodar et al 2001). In addition, children normally associate the site of needle injection with pain, resulting in a drop of the rate of compliance. Thus, there is an urgent need for the development of a new generation of safer vaccines that can be effectively administered by simple, economical and practical immunization procedures (Partidos et al 2002).

Topical immunization, i.e. non-invasive vaccination onto the skin, provides a robust and novel approach of vaccination. It allows for vaccination by individuals without any medical training, and makes widespread vaccination more cost effective and feasible. The approach is unique in the sense that immunocompetent Langerhan's cells (dendritic cells) are found in abundance below the epidermis and are aligned specifically along the minute pores in dermis through which extracorporal pathogens are likely to invade the body. Apart from these epidermal cells, antigen presenting cells and migratory T-lymphocytes are also present. All these cells are collectively known as skin-associated lymphoid tissue and constitute the skin immune system. These cells altogether function in association with lymph nodes and are responsible for the generation of cellular and humoral immune responses (Babiuk et al 2000; Singh et al 2002; Gupta et al 2004). However, several challenges are encountered in delivering large molecular weight antigens across the skin due to size, charge and other physicochemical properties. Stratum corneum acts as the major barrier for transfer of antigens

to the dermal and epidermal sites. To overcome these problems a number of approaches, including encapsulation of antigens within suitable carrier systems, have been explored for their efficient delivery through the topical route. Vesicular carrier systems liposomes, niosomes and transfersomes have been widely advocated for topical delivery of bioactives. They are able to cross the skin barrier either via penetration of stratum corneum or entry via the pilosebaceous route upon topical application (Schreier & Bouwstra 1994; Fang et al 2001; Bouwstra & Honeywell-Nguyen 2002). Recently, tetanus toxoid-loaded liposomes, niosomes and transfersomes have been prepared in our laboratory for non-invasive topical immunization (Gupta et al 2005a, b). The low cost, high purity, content uniformity, greater stability and ease of storage of non-ionic surfactants have presented niosomes as better alternatives to liposomes. These vesicles appear to be similar in terms of their physical properties to liposomes, being prepared in the same way and under a variety of conditions forming unilamellar or multilamellar structures (Yoshioka et al 1994; Uchegbu & Vyas 1998).

It has been well established that Langerhan's cells (the major antigen presenting cells present beneath the skin) like other dendritic cells express mannose receptors on their surfaces (Kery et al 1992; Avrameas et al 1996). A number of studies have been reported showing that the mannosylated carriers could be selectively presented to dendritic cells and such systems displayed superior immunoadjuvant action to conventional ones (Garcon et al 1988; Sugimoto et al 1995; Cui & Mumper 2002; Copland et al 2003).

It has been envisaged that coating the niosome surface with the polysaccharide mannan will not only facilitate the delivery of encapsulated antigen across the stratum corneum, but additionally serve to present it selectively to these antigen presenting cells for elicitation of strong immunological response. Thus in this study we have investigated antigen-loaded and mannan-coated niosomes for their potential in topical immunization as a vehicle as well as an adjuvant.

Materials and Methods

Materials

Sorbiton monostearate (Span 60), sorbiton trioleate (Span 85) and palmitoyl chloride were procured from Fluka, Switzerland. Cholesterol, stearylamine, mannan, Sephadex G-100, BSA, Triton X-100, concanavalin A and horseradish peroxidase (HRP) labelled anti-rat IgG, IgG1 and IgG2a were purchased from Sigma Chemicals Co. (USA). Substrate tetramethyl benzidine-hydrogen peroxide (TMB-H_2O_2) and bicinchoninic acid (BCA) protein estimation kits were obtained from Genei Banglore, India. All other chemicals and solvents used were purchased from local suppliers and were of analytical grade unless otherwise mentioned.

Preparation of niosomes

Niosomes were prepared by the reverse-phase evaporation method (Kiwada et al 1985). Surfactant (Span 60/Span 85), cholesterol and stearylamine in a molar ratio 6:3:1 were dissolved in diethyl ether, followed by emulsification with aqueous solution of BSA (1 mg mL^{-1} in phosphate-buffered saline; PBS, pH 7.4) by probe sonication (Soniweld, India) for 5 min at 40 Kc s^{-1}. The organic solvent was evaporated in a rotary flash evaporator at 40°C under reduced pressure (260–400 mmHg). The lipid gel so formed was collapsed and transformed into a fluid with continual vigorous mechanical agitation using a vortex mixer followed by addition of warm PBS (pH 7.4) to hydrate the vesicles. Unentrapped BSA was removed by passing the niosomal suspensions through a Sephadex G-100 mini column and centrifugation at 3000 rev min^{-1} for 3 min (Fry et al 1978; New 1990).

Coating with mannan

Niosomes were coated with O-palmitoyl mannan (OPM) following the procedure developed in our laboratory (Vyas et al 2000). OPM was synthesized by esterification of mannan in dimethyl formamide under catalytic conditions (Sunamoto et al 1985; Vyas et al 2000). For coating, 2 mL formulation was incubated and gently stirred with 1 mL OPM dispersion (in PBS, pH 7.4) at room temperature. After completion of coating the excessive unbound polysaccharide was removed by gel filtration using a Sephadex G-100 minicolumn. The two process variables, total lipid to OPM ratio and optimum incubation time for coating, were optimized by measuring the change in zeta potential of the dispersion. For optimization of total lipid to OPM ratio, formulations with different ratios were prepared and incubated for a fixed time-period of 24 h and zeta potential was measured (Zetasizer, Malvern Instruments Co., UK). The optimum ratio was determined at which no significant change in zeta potential was recorded on further increasing the lipid to OPM ratio. Similarly, for optimization of incubation time, the formulations with optimum lipid:OPM ratio were prepared and incubated for different time periods and zeta potential was measured. After completion of coating no significant change in zeta potential was recorded.

In-vitro characterization

Developed formulations were characterized before and after surface ligand anchoring. Formulations were evaluated for their shape and morphology by phase contrast microscopy (Leitz-biomed, Germany) and transmission electron microscopy (TEM) (Philips, Japan). For determination of entrapment efficiency vesicles were lysed using a minimum amount of Triton X-100 (0.5% v/v) and the liberated antigen (BSA) was estimated using the BCA method after suitable dilution. Vesicle size and size distribution studies were carried out using a laser diffraction based particle size analyser (CILAS, 1064, France).

In-vitro ligand binding specificity

The prepared OPM-coated niosomal systems were assessed for in-vitro ligand-specific activity by concanavalin

A (Con A) agglutination assay as described by Copland et al (2003), with appropriate modification. A 200-μL sample of the original niosomal dispersion (plain or OPM coated) was diluted 10-times with PBS (pH 7.4) and 1 mL Con A (1 mg mL^{-1}) in PBS (pH 7.4) with 5 mM calcium chloride, and 5 mM magnesium chloride was added to it. The increase in turbidity at 550 nm was monitored spectrophotometrically (Shimadzu 1601 DB UV/vis spectrophotometer, Japan) for 2 h.

Immunization experiments

Albino rats (100–150 g) were used for in-vivo studies. Animals were housed in groups of five with free access to food and water. The study was carried out under the guidelines compiled by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animal, Ministry of Culture, Government of India) and all the study protocols were approved by Institutional Animal Ethics Committee of our university. The hairs of the rats were shaved and the skin was carefully wiped with 70% ethanol to remove the outermost keratin layer to ensure an optimal interaction of the applied formulation with the stratum corneum (van den Bergh et al 1999). For immunization various formulations (alum-adsorbed BSA, plain or OPM-coated niosomes) in the form of an aqueous suspension in a dose equivalent to 100 μg BSA were applied with gentle rubbing for three consecutive days, followed by a booster dose three weeks after the first dose. To serve as the standard for comparison, a group of animals were immunized with a single intramuscular (i.m.) dose of alum-adsorbed BSA followed by a booster dose after three weeks. In addition, a physical mixture of empty niosomes and BSA solution in equivalent doses was applied topically to a group following the above set protocol, to study the permeation enhancer effect of the surfactant. Blood samples were collected from the retro-orbital plexus of rats at biweekly intervals for eight weeks after immunization. Serum was obtained by centrifugation of blood samples and kept at -40°C until tested by ELISA.

Analysis of the immune response

Antibody responses in immunized animals were monitored using a microplate ELISA procedure. Microtitre plates (Nunc-Immuno Plate Fb 96 Mexisorp, NUNC) were coated with 100 μL/well 1% BSA solution in PBS (pH 7.4) and incubated overnight at 4°C. The plates were washed three times with PBS-Tween 20 (0.05% v/v) (PBST). The serum samples were serially diluted with PBS and 100 μL of each sample was added to each well of the coated ELISA plates. The plates were incubated for 1 h at room temperature and washed three times with PBST. Peroxidase-labelled goat anti-rat IgG, IgG2a or IgG1 (100 μL) was added to each well. The plates were covered and after incubation for 1 h at room temperature the washing was repeated. Substrate solution (TMB-H_2O_2, 100 μL/well) was added followed by addition of 50 μL 2 M H_2SO_4 after 20 min to stop the reaction. The intensity of the developed colour after 15 min was measured at 450 nm using a plate reader (Labsystems, Finland). End point titres were expressed as the log of the reciprocal of the last dilution, which gave an optical density (OD) at 450 nm above the OD of negative controls. IgG2a/IgG1 fraction was calculated from respective log values of end point titres.

Statistical analysis

The results were expressed as mean $\pm$ standard deviation (s.d.). One way analysis of variance (ANOVA) followed by post hoc test (Tukey's test) was used to evaluate the effect of lipid to OPM ratio or incubation time and zeta potential ($n = 6$). The effect of incubation time with lectin and niosome type on optical density was analysed using repeated measures ANOVA followed by Tukey's test ($n = 6$). Analysis of serum IgG titres achieved after different time intervals following immunization using different formulations was also performed using two-way ANOVA followed by Tukey's test ($n = 5$). One-way ANOVA followed by Tukey's test was used to analyse the serum IgG2a/IgG1 ratio ($n = 5$). Statistical significance was designated as $P < 0.05$.

Results and Discussion

Preparation and in-vitro characterization

Niosomes were prepared by the reverse-phase evaporation method. This method has been reported to encapsulate large hydrophilic macromolecules with relatively high entrapment efficiencies (Szoka & Papahadjopoulos 1978), and it has been associated with the highest skin penetrating capability (Cevc et al 1996). Span 60 (sorbitan monostearate) and Span 85 (sorbitan trioleate) were used as non-ionic surfactants for niosome preparation. These surfactants were selected due to the extreme differences in their chemical structures, molecular weight and lipophilicity. Span 60 ($C_{24}H_{46}O_6$) is a creamy yellow solid with molecular weight 431 Da and HLB value 4.7. Span 85 ($C_{60}H_{108}O_8$) has a molecular weight 958 Da and HLB value 1.8, and it is a yellow viscous liquid with relative density 1.01 g mL^{-1}. Initial experiments were conducted to optimize the procedure of ligand anchoring. Since mannan is an extremely hydrophilic polysaccharide, it cannot be simply coated with a lipid surface of vesicles by simple incubation. It was necessary to chemically modify the mannan by conjugating it to a hydrophobic anchor, which allowed the polysaccharide to interdigitate with the niosomal membrane. During the coating process the hydrophobic palmitoyl tail of OPM integrated with the lipoidal niosomal membrane projecting from the hydrophilic mannose residues towards the bulk aqueous phase. Moreover, OPM possessed a negative charge, while niosomes were positively charged due to the stearylamine, which further facilitated the adsorption process and resulted in a reduction in the zeta potential of the dispersion, which was used to optimize the process variables (lipid-to-OPM ratio and incubation

time). For the Span 60-based niosomes the initial positive value of the zeta potential decreased on addition of anionic ligand OPM and approached towards a minimum value at 5:1 lipid:ligand weight ratio (Figure 1a). It apparently related to the extent of the masking of the surface charge by OPM. On further addition of OPM especially beyond this optimum ratio (5:1 w/w) no significant change in the zeta potential occurred ($P < 0.05$). It indicated that at an optimal 5:1 weight ratio of lipid:ligand, the integration of OPM with the surfactant bilayer membrane occurred at saturation level. For optimization of incubation time the formulations using the optimum lipid-to-OPM ratio were prepared and incubated for different incubation time periods (0, 1, 2, 3, 4, 6, 8, 12 and up to 24 h) and the change in zeta potential was recorded (Figure 1b). The zeta potential values declined steeply from their initial values, which might have been attributed to the charge quenching of the surface associated with free OPM. With a longer incubation time (beyond 4 h), the amount of residual free OPM decreased and the change in zeta potential was not significant ($P < 0.05$). This showed that at the end of 4 h, the interaction and interdigitation of added OPM could have completed.

Like Span 60-based formulations, the total lipid:OPM ratio 4:1 w/w was considered as the optimum for OPM coating of Span 85-based niosomes as the measured zeta potential values were found to be least variant ($P < 0.05$) after this ratio (Figure 2a). Similarly, no significant change in zeta potential ($P < 0.05$) was observed after 6 h incubation (Figure 2b). Thus, 4:1 weight ratio of total lipid-to-OPM and 6 h incubation time were found to be optimum for complete coating in the case of the Span 85-based niosomal formulations.

Morphologically, the vesicles were spherical and multilamellar in shape. However, under the microscope OPM-coated niosomes were opaque, probably due to a distinct polysaccharide coat. The shape of the vesicles was identical irrespective of the lipid composition and lipids used in the preparation. Percent BSA encapsulation efficiency was found to be $44.3 \pm 3.8\%$ and $41.2 \pm 3.2\%$ for Span 60- and Span 85-based plain niosomes, respectively. Span 60 is a saturated surfactant with a higher phase transition temperature and forms a more stable, rigid bilayer with low fluidity (less leaky) compared with unsaturated Span 85 surfactant, which might have accounted for the relatively higher encapsulation efficiency (statistically significant, $P < 0.05$) as compared with Span 85-based formulations (Yoshioka et al 1994). Encapsulation efficiency was slightly reduced to $40.6 \pm 3.3\%$ (Span 60) and $36.5 \pm 3.5\%$ (Span 85) for OPM-coated niosomes. The effect was statistically significant ($P < 0.05$) and might have accounted for the removal of surface-associated antigen and leakage of some encapsulated antigen

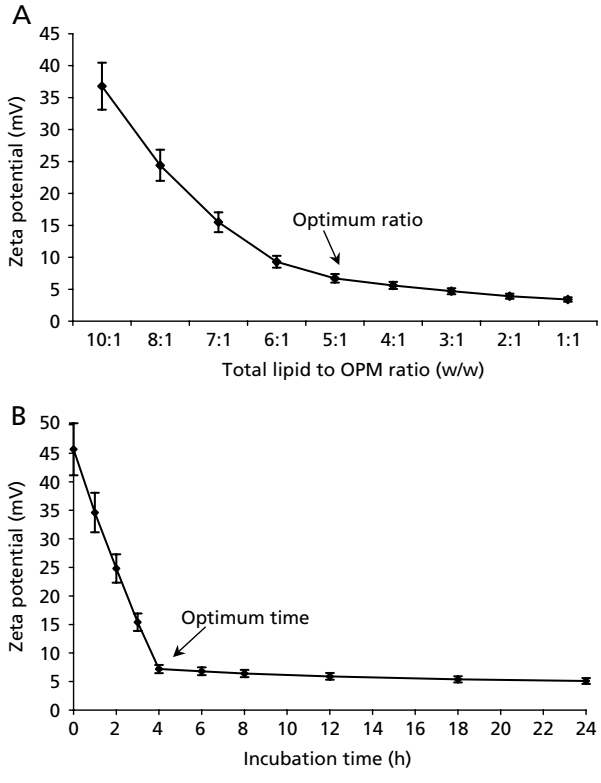

Figure 1 Optimization of coating process parameters for Span 60-based niosomes. A. Total lipid to OPM weight ratio. B. Incubation time. Values are expressed as mean ± s.d. (n = 6).

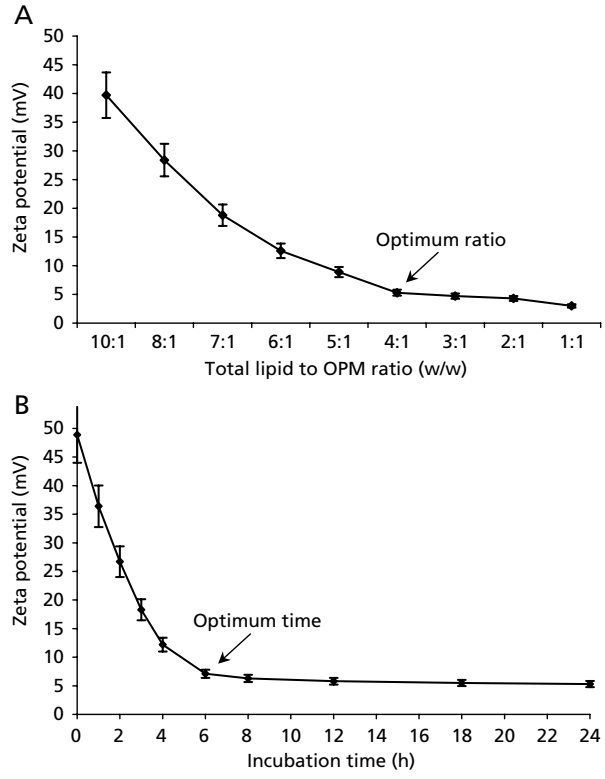

Figure 2 Optimization of coating process parameters for Span 85-based niosomes. A. Total lipid to OPM weight ratio. B. Incubation time. Values are expressed as mean ± s.d. (n = 6).

during the coating process. Mean vesicle size was found to be $2.52 \pm 0.23\,\mu$m (Span 60) and $1.86 \pm 0.25\,\mu$m (Span 85). The results were in accordance with the finding that increasing the lipophilicity of the surfactant monomer led to smaller vesicles, a result which might have been anticipated since surface free energy decreases with increasing hydrophobicity (Yoshioka et al 1994). The mean size of OPM-coated vesicles was $2.97 \pm 0.27\,\mu$m (Span 60) and $2.34 \pm 0.31\,\mu$m (Span 85). OPM-coated niosomes were relatively larger in size as compared with the uncoated niosomes due to the surfacial OPM coat.

The presence of accessible mannose residues on the vesicle surface was confirmed by the in-vitro Con A agglutination assay. Con A is one of the well-investigated lectins and is known to specifically bind with mannose, fructose and glucose residues (Rademacher et al 1988; Palomino 1994). A dramatic increase in the turbidity as monitored by optical density at 550 nm was observed in the case of the dispersion containing OPM-coated niosomes following exposure to Con A. In contrast, plain niosomes did not show any significant change ($P < 0.05$) in turbidity following exposure to lectin (Figure 3a, b). The results suggested that mannan retained the binding specificity towards the lectins even after chemical modification and anchoring onto the niosome's surface, and they were oriented towards the aqueous bulk and were available for such interaction.

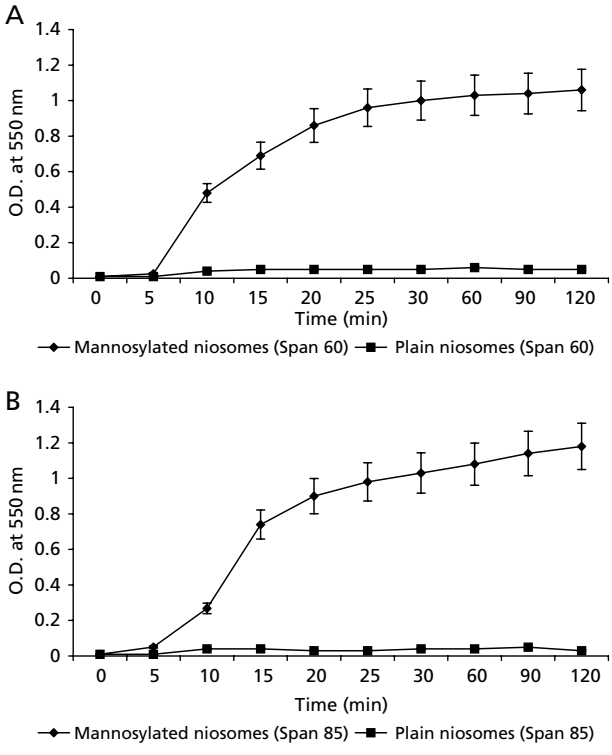

Figure 3 In-vitro ligand specificity of mannosylated niosomes by Con A-induced aggregation method. A. Span 60-based niosomes. B. Span 85-based niosomes. Values are expressed as mean ± s.d. (n = 6).

Serum IgG level following topical immunization

The serum IgG profile was determined for all experimental groups after 2, 4, 6 and 8 weeks of primary immunization to evaluate systemic immune response induced by the developed formulations (Table 1). The serum IgG titre achieved after 4 weeks topical immunization using various BSA-loaded niosomal formulations, Span 60 (plain), Span 85 (plain), Span 60 (OPM coated) and Span 85 (OPM coated), were 3.8 ± 0.3, 4.3 ± 0.4, 4.9 ± 0.6 and 5.3 ± 0.6, respectively. The intramuscular standards of BSA produced the highest titre value (6.4 ± 0.7) after four weeks. Very low titre, 1.2 ± 0.2 was obtained with topically applied alum-adsorbed BSA (control). It might have been due to the fact that plain antigen fails to cross the intact skin barrier due to high molecular weight, negative charge and hydrophilic nature.

It has been inferred from the results that topical immunization using niosomal formulations exhibited significantly greater antibody titre ($P < 0.05$) in serum as compared with topical control, but the response was considerably lower (statistically significant $P < 0.05$) when compared with the intramuscular standard. There are a number of mechanisms that have been proposed for topical delivery of bioactives via lipid based carriers. For niosomes it was thought that the surfactants served as penetration enhancers by raising the fluidity and reducing the barrier property of the stratum corneum (Sarpotdar & Zatz 1986; Valjakka-Koskela et al 1998). However, topical application of a physical mixture of empty niosomes and BSA solution did not produce good results. The titre value achieved in this case (1.8 ± 0.2 after 4 weeks) was comparable with that obtained with topical BSA control and was significantly lower than the niosomal formulations

Table 1 Serum IgG profile of rats immunized with different formulations

Formulation	Serum IgG levels (log)			
	2 weeks	4 weeks	6 weeks	8 weeks
Alum-adsorbed BSA (i.m.) (standard)	4.9 ± 0.5	6.4 ± 0.7	6.2 ± 0.5	5.8 ± 0.6
Alum-adsorbed BSA topical (control)	1.1 ± 0.1	1.2 ± 0.2	1.1 ± 0.2	1.0 ± 0.1
Empty niosomes + BSA	1.5 ± 0.1	1.8 ± 0.2	1.6 ± 0.2	1.3 ± 0.2
Plain niosomes (Span 60)	2.7 ± 0.2	3.8 ± 0.3	3.4 ± 0.3	3.0 ± 0.2
Mannosylated niosomes (Span 60)	3.7 ± 0.3	4.9 ± 0.6	4.5 ± 0.5	4.1 ± 0.5
Plain niosomes (Span 85)	3.1 ± 0.4	4.3 ± 0.4	4.0 ± 0.3	3.6 ± 0.3
Mannosylated niosomes (Span 85)	4.0 ± 0.4	5.3 ± 0.6	5.1 ± 0.6	4.8 ± 0.5

Values are expressed as mean ± s.d. (n = 5).

($P < 0.05$). It is therefore suggested that factors other than the permeation enhancer effect of surfactants were involved in the topical delivery of the bioactive. Studies have shown that mixing of liposomes with skin lipids in the intercellular layer could be one mechanism contributing to the enhancement of drug permeation (Weiner et al 1989). The same phenomenon was observed with niosomes. Accordingly, vesicle adsorption and fusion onto the surface of the skin disrupts the membrane (barrier) property of the stratum corneum and leads to a high thermodynamic activity gradient of bioactive-stratum corneum interface (Schreier & Bouwstra 1994). Vesicles are also reported to follow the pilosebaceous route for entry of macromolecules including interferons, monoclonal antibodies and DNA, thus bypassing the stratum corneum barrier (Touitou et al 1994; Weiner 1998).

Further, the significantly greater response of mannan-coated niosomes as compared with plain niosomes ($P < 0.05$) could be attributed to targeting of mannan to mannose receptors on the dendritic cells (Langerhan's cells) and other antigen presenting cells of skin-associated lymphoid tissue that are located at the basal layer of the epidermis and cover more than 20% surface area (Kery et al 1992; Avrameas et al 1996). The results were in accordance with the findings of Watabe et al (2001) and Cui & Mumper (2002) who developed mannosylated liposomes and mannosylated nanoparticles for better humoral and cellular immune response upon topical genetic immunization using respective mannosylated carrier. In addition to this, mannan could elicit some intrinsic immunogenic effect that could considerably aggravate the immunogenic response of antigen through adjuvant effect (Garcon et al 1988).

The results showed that Span 85-based formulations produced significantly better immune response as compared with the Span 60-based formulations upon topical application ($P < 0.05$). Span 85 is an unsaturated surfactant and the packing nature of unsaturated lipids/surfactants chains are reported to change the fluidity of the stratum corneum and facilitate the skin permeation of bioactives to a higher extent as compared with saturated lipids/surfactants (Valjakka-Koskela et al 1998; Valenta et al 2000).

Serum IgG2a/IgG1 ratio

Serum IgG2a/IgG1 ratio was measured after 4-weeks immunization using various formulations to determine the extent of humoral and cellular immune response (Table 2). It has been reported that the nature of antigen presenting cells that present the antigen to specific T helper cells will favour the isotype and magnitude of the B cell antibody response (Constant & Bottomly 1997; Morokata et al 2000). Dendritic cells are considered as major antigen presenting cells and are widely distributed on the epithelial barriers at different sites of the body. They are capable of processing the antigen by MHC I and MHC II pathways, depending upon the nature of the recognized antigen. Antigen processed by MHC I molecules is presented to Th1 cells to elicit cellular immune response, while that processed via MHC II molecules is presented to Th2 cells and elicits humoral response. Thus, different antigen-loaded formulations could be processed in different ways and presented to a specific T helper cell subpopulation, which could originate differences in the specific serum IgG2a/IgG1 antibody production (Banchereau et al 2000). IgG1 is an antibody associated with inflammatory and mast cells or eosinophil responses, while IgG2a is able to act as an opsonin, activates the complement and binds to macrophages and enhances the phagocytic response (Lubeck et al 1985). Studies have revealed that switching to IgG2a is induced by IL-12 and IFN-γ (Th1 dependent cytokines) and inhibited by IL-4, IL-5 and IL-10 (Th2 dependent cytokines). Thus the ratio of IgG2a/IgG1 gives an indirect indication about the extent of cellular and humoral immune response (Constant & Bottomly 1997).

Table 2 Serum IgG2a/IgG1 ratio after four weeks of immunization using different formulations

Formulation	Serum IgG2a/IgG1 ratio
Alum-adsorbed BSA (i.m.)	0.45 ± 0.03
Plain niosomes (Span 60)	0.52 ± 0.09
Mannosylated niosomes (Span 60)	0.68 ± 0.06
Plain niosomes (Span 85)	0.56 ± 0.06
Mannosylated niosomes (Span 85)	0.71 ± 0.07

Values are expressed as mean $\pm$ s.d. ($n = 5$)

It is evident from Table 2 that all developed niosomal formulations were capable of eliciting a combined serum IgG2a/IgG1 response, with predominance of IgG1 response (humoral response). The differences were statistically insignificant ($P < 0.05$). The results were consistent with the findings that processing and presentation of particulate antigens by MHC class I and II by dendritic cells and macrophages stimulated Th1 and Th2 lymphocyte subpopulations, while soluble antigens were exclusively presented by MHC II molecules and stimulated Th2 response (Morokata et al 2000; Singh et al 2004). This reflected that topical application of antigens in mannosylated niosomes could elicit both humoral and cellular immune response. However, more studies concerning T lymphocyte proliferation assays and cytokine production need to be conducted to completely characterize the immune response elicited by this system.

Conclusion

It could be concluded from the results that the prepared system, i.e. mannosylated niosomes, could be used as effective carrier and adjuvant for non-invasive, non-parenteral immunization through the topical route. The proposed system was capable of eliciting humoral and cellular immune response and could be a promising carrier system for a number of antigens other than BSA. It could overcome the disadvantages of classical invasive methods of vaccination and was simple, economical, stable, painless

and potentially safe. With the identification of such type of carrier system that has the potential to elicit systemic immunity following topical application, a new way of low cost administration of potential vaccines could be developed, which would be of particular use in developing countries. However, elaborative studies and clinical trials with selected antigens would be required.

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