JPP
Journal of
Pharmacy and Pharmacology

JPP 2005, 57: 295–301
© 2005 The Authors
Received August 7, 2004
Accepted November 17, 2004
DOI 10.1211/0022357055515
ISSN 0022-3573

Tetanus toxoid-loaded transfersomes for topical immunization

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Abstract

Topical immunization is a novel immunization strategy by which antigens and adjuvants are applied topically to intact skin to induce potent antibody and cell-mediated responses. Among various approaches for topical immunization, the vesicular approach is gaining wide attention. Proteineous antigen alone or in combination with conventional bioactive carriers could not penetrate through the intact skin. Hence, specially designed, deformable lipid vesicles called transfersomes were used in this study for the non-invasive delivery of tetanus toxoid (TT). Transfersomes were prepared and characterized for shape, size, entrapment efficiency and deformability index. Fluorescence microscopy was used to investigate the mechanism of vesicle penetration through the skin. The immune stimulating activity of these vesicles was studied by measuring the serum anti-tetanus toxoid IgG titre following topical immunization. The immune response was compared with the same dose of alum adsorbed tetanus toxoid (AATT) given intramuscularly, topically administered plain tetanus toxoid solution, and a physical mixture of tetanus toxoid and transfersomes again given topically. The results indicated that the optimal transfersomal formulation had a soya phosphatidylcholine and sodium deoxycholate ratio of 85:15%, w/w. This formulation showed maximum entrapment efficiency ($87.34 \pm 3.81\%$) and deformability index (121.5 ± 4.21). An in-vivo study revealed that topically administered tetanus toxoid-loaded transfersomes, after secondary immunization, elicited an immune response (anti-TT-IgG) comparable with that produced by intramuscular AATT. Fluorescence microscopy revealed the penetration of transfersomes through the skin to deliver the antigen to the immunocompetent Langerhans cells.

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Funding and acknowledgements: The Authors thank the Serum Institute of India, Pune, for providing the gift sample of tetanus toxoid. P. N. Gupta thanks the Indian Council of Medical Research (ICMR), New Delhi, India, for providing financial assistance to carry out the research work. The help and facilities provided by the Head, Department of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar, M.P., India are duly acknowledged.

Introduction

Vaccination represents one of the most cost-effective preventive measures against illness and death from infectious diseases. Except for the oral polio vaccine, all EPI (Expanded Programme on Immunization) vaccines are given by an invasive method using needles and syringes. Thus immunization requires trained medical personnel, is expensive, it may lead to injection site reaction, and in some cases to infection by transmitting blood-borne pathogens such as hepatitis B or HIV (Simonsen et al 1999). In addition, the traumatic nature of conventional immunization practice reduces the patient compliance. Hence, a non-invasive immunization practice would be safer, more acceptable and more suitable for mass use (Levine 2001; Gupta et al 2004).

Non-invasive or needle-free immunization via the skin is an attractive alternative that reduces the potential problems associated with needle injection. The skin is an immunologically active site and a promising vaccination route (Chen et al 2001). Transcutaneous immunization can be achieved by simple application of vaccine to the skin surface. This approach combines the advantages of needle-free delivery while targeting the immunologically rich milieu of the skin. Vaccination through the skin may be particularly advantageous as the immunocompetent Langerhans cells are found in abundance along the transdermal penetration pathways and these cells are aligned specifically along the minute pores through which pathogens are likely to invade the body. Langerhans cells are found in close proximity to the stratum corneum and represent a network of immune cells that underlie 25% of the skin's total surface area (Paul & Cevc 1995; Glenn et al 2000). Epidermal Langerhans cells play a vital role in antigen presentation to $CD4^+$ T cells

(Kupper 1990). These cells bind cutaneously encountered antigen and then process it. Along with processed antigen, Langerhans cells migrate from the epidermis into lymphatic vessels and finally into regional lymph nodes. Differentiation of Langerhans cells into dendritic cells occurs during this process and the dendritic cells offer the antigen to naive $CD4^+$ T cells that have entered the lymph nodes through the high endothelial venules (Paul et al 1998).

Topical antigen delivery using a suitably designed micro-invader is a novel approach, which would resemble the normal pathway of body infection and would result in a robust immune response. During its passage through the intact skin, the antigen carrier would encounter Langerhans cells and dendritic cells, and would start antigen presentation by these cells, which are found in abundance in the skin and facilitate communication with rest of the body. Thus, topical antigen delivery has the potential to produce a local as well as a systemic immune response. Among the various approaches for topical immunization, namely physical, chemical and vesicular, the latter is gaining wide attention. Vesicular carriers i.e. transfersomes, liposomes, niosomes etc., elicit immune responses by different mechanisms. Some lipids directly lower the skin permeability barrier, which resides primarily in the stratum corneum. Hence the specially designed lipid vesicles could be a better module for topical delivery of proteineous antigens.

Various types of surfactants have been used for the preparation of non-ionic vesicles such as polyglycerol alkyl ethers (Handjani-vila et al 1979; Ballie et al 1985), glucosyl-dialkyl ethers (Kiwada et al 1985) and polyoxy ethylene ethers (Hofland et al 1991). The most recent development in vesicle design for transdermal bioactive delivery is the use of elastic vesicles, transfersomes, that differ from conventional niosomes and liposomes by their characteristic fluid membrane with high elasticity. This feature enables transfersomes to squeeze themselves through intercellular regions of the stratum corneum under the influence of a transdermal water gradient (Cevc et al 1998).

In this study, transfersomes were used for non-invasive delivery of tetanus toxoid (TT). Transfersomes were prepared and characterized for their size, shape and entrapment efficiency. The extrusion rate of vesicles was measured to estimate the value of elasticity (deformability index). The immune response elicited by topically-applied tetanus toxoid-loaded transfersomes was compared with intramuscularly administered alum adsorbed tetanus toxoid (AATT), topically administered plain tetanus toxoid solution and a topically given physical mixture of tetanus toxoid and transfersomes, by measuring IgG antibody titre. Fluorescence microscopy was used to investigate the mechanism exploited by this vesicular carrier to deliver the tetanus toxoid.

Materials and Methods

Materials

Soya phosphatidylcholine, sodium deoxycholate, Sephadex G-150, and 6-carboxyfluorescein (6-CF) were purchased from Sigma, USA. Protein estimation kit (by BCA method) and ELISA kit (including horseradish peroxidase conjugated anti-rat IgG and substrate for ELISA) were purchased from Genei, Bangalore, India. All solvents used were of analytical grade. Tetanus toxoid (TT) was obtained as gift sample from Serum Institute of India, Pune. The tetanus toxoid solution contained 3600 Lime flocculation mL^{-1} ($Lf\,mL^{-1}$) and a protein concentration of $9.0\,mg\,mL^{-1}$.

Preparation of transfersomes

Transfersomes were prepared by a method described by Paul et al (1998) with slight modifications. In brief, 2.0 mL ethanolic solution of soya phosphatidylcholine was mixed with sodium deoxycholate (95:5; 90:10; 85:15; 80:20 and 75:25% w/w) in 3.0 mL 0.2 M phosphate buffer (pH 6.5) containing tetanus toxoid ($95\,Lf\,mL^{-1}$). The obtained suspension was pushed through a series of 0.45-, 0.22-, 0.10- and 0.05-μm polycarbonate membrane filters (Nucleopore, The Netherlands). Similarly, 6-CF-loaded transfersomes were prepared with optimum lipid composition to study penetration behaviour of transfersomes through the skin layers.

Vesicle morphology and size analysis

Prepared transfersomes were characterized for their vesicle shape using transmission electron microscopy (JEM-200 CX, JEOL, Tokyo, Japan). Phosphotungstic acid 1% (PTA) was used as the negative stain for the transmission electron microscopy. Particle size of the prepared vesicular system was measured by photon correlation spectroscopy with an Autosizer II C apparatus (Malvern Instruments, UK).

Entrapment efficiency

Prepared transfersomes were taken and separated from the free (un-entrapped) antigen by a Sephadex G-150 minicolumn using a centrifugation technique (Fry et al 1978). The method was repeated thrice with a fresh syringe packed with gel each time. The fraction that was finally collected was free from un-entrapped antigen. The vesicular fraction was added with a minimum amount of Triton X-100 (0.5% w/v) to disrupt the vesicles. The liberated antigen was estimated by BCA (bicinchoninic acid) protein assay and percentage antigen entrapment was determined (Table 1).

Table 1 Effect of lipid-to-surfactant ratio on the entrapment efficiency and deformability index of tetanus toxoid-loaded transfersomes

Formulation code	SPC:SDC (%w/w)	Entrapment efficiency (%)*	Initial size (nm)*	Deformability index*
TD1	95:5	84.72 ± 3.84	172 ± 8	88.7 ± 2.91
TD2	90:10	85.75 ± 4.79	181 ± 7	107.4 ± 4.32
TD3	85:15	87.34 ± 3.81	188 ± 9	121.5 ± 4.21
TD4	80:20	82.52 ± 3.89	191 ± 9	105.0 ± 3.92
TD5	75:25	81.14 ± 4.87	195 ± 10	103.4 ± 3.12

*All values are expressed as mean ± s.d. (n = 4). SPC, soya phosphatidylcholine; SDC, sodium deoxycholate.

Measurement of elasticity value (deformability index)

Elasticity of the bilayer was estimated by extrusion measurement (Bergh et al 2001). Briefly, the vesicles were extruded through a 50-nm pore size polycarbonate filter (Nucleopore, The Netherlands) at constant pressure. The elasticity of the vesicle was expressed in terms of deformability index (Table 1). This was determined using the formula:

$$j\ (r_v/r_p)^2$$

where j is the weight of suspension that was extruded over 10 min through a 50-nm pore size polycarbonate filter, r_v is the size of vesicle, and r_p is the pore size of the barrier.

In-vitro skin permeation experiment

The permeation of tetanus toxoid-loaded transfersomes, plain tetanus toxoid solution, and a physical mixture of tetanus toxoid and transfersomes through the skin was determined by using a locally fabricated Franz-diffusion cell. Before use the skin was inspected for any damage using microscopy after staining with haematoxylin and eosin. The nude rat skin was mounted on the receptor compartment with the stratum corneum side facing upward into the donor compartment. The formulation containing 40 μg tetanus toxoid was applied on the skin in the donor compartment. The receptor medium was 5 mL 0.2 M phosphate-buffered saline (PBS) (pH 6.5). The receptor compartment was maintained at 37°C with magnetic stirring at 500 rev min^{-1}. At appropriate intervals 200-μL receptor medium was withdrawn and immediately replaced with an equal volume of fresh receptor solution. The samples from the receptor medium were analysed by the BCA method.

Fluorescence microscopy

6-CF-containing transfersomes and plain 6-CF solution were applied topically to the shaved skin of albino rats. After 4 h the rats were killed and the skin was removed. Microtomy was performed and ribbons of sections (thickness 6 μm) were fixed onto glass slides using egg albumin as the fixative. The sections were viewed under a fluorescence microscope (Leica wild MP 582, Switzerland).

Immunization

Albino Wistar rats (8–12-weeks old) were used for the immunization studies. Each group contained eight animals. The animals were kept under standardized conditions at the Pharmaceutical Departmental Animal Facility of the Dr. H.S. Gour University, Sagar, M.P., India. The study was carried out under the guidelines compiled by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Culture, Govt. of India. The animals were carefully shaved on their dorsum and rested for 24–48 h. The skin was carefully wiped with 70% ethanol before application of the vesicular formulation. For immunization two protocols were used. Protocol 1, a single immunization on day 0 without any booster dose. Protocol 2, a single immunization on day 0 followed by a booster dose on day 28 with the same formulation and with the same dose. Tetanus toxoid-loaded transfersomes, plain tetanus toxoid solution, and a physical mixture of tetanus toxoid and transfersomes equivalent to 10 Lf tetanus toxoid was applied to the shaved skin over a $2 \times 2\,cm^2$ area and left to dry. The immune response was compared with same dose of alum adsorbed tetanus toxoid (AATT) given intramuscularly. Blood samples were withdrawn on days 14, 28, 42, 56 and 90 through the retro-orbital plexus vein in the eye. The collected blood samples were allowed to clot and then centrifuged to separate the serum, which was stored at −20°C until analysis.

Determination of anti-tetanus toxoid antibody

Antibody levels against tetanus toxoid were determined by ELISA as described by Esparza & Kissel (1992). Tetanus toxoid 100 μL (10 μg mL^{-1} in PBS pH 7.4) was coated to each well of a Nunc-Immuno plate. The plate was incubated at 4°C overnight. The plate was then washed three times with PBS–Tween 20 (0.05% v/v). To each well was added 100 μL 2% BSA, the plate was incubated for 2 h at room temperature and washed three times with PBS–Tween 20 (0.05% v/v). A diluted serum sample (100 μL) was added to each well and incubated for 2 h at room temperature. The plate was washed three times with PBS–Tween-buffer. Diluted horseradish peroxidase conjugated antiglobulin specific anti-rat IgG (500-times dilution) 100 μL was added to each well and incubated for 2 h. The plate was again washed three times with PBS–Tween 20 (0.05% v/v) and then 100 μL substrate solution 3, 3′, 5, 5′ tetramethyl benzidine (20-times dilution) containing hydrogen peroxide was added to each well. The plate was incubated in darkness at room temperature for 15 min. The reaction was stopped by adding 50 μL 2 M H_2SO_4 to each well. The absorbance was measured at 450 nm using a microplate ELISA reader (Lab Systems Multiscan, Finland). Results are shown in terms of log reciprocal end point dilution.

Statistical analysis

The effect of the formulations on the entrapment efficiency, initial size and deformability index were analysed by Kruskal–Wallis test using SYSTAT version 10.2.01 software and differences were considered statistically significant at $P < 0.5$. The antibody titres and in-vitro cumulative antigen permeation were analysed statistically by one-way analysis of variance followed by post-hoc Tukey's test. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Transfersomes preparation and characterization

Tetanus toxoid-loaded transfersomes were prepared as described by Paul et al (1998). The method was mild, not

involving harsh conditions, thus it was found suitable for the preparation of carriers for antigen delivery. Good encapsulation efficiency was achieved with this method and under an electron microscope these carriers appeared as unilamellar vesicles.

To cross the intact mammalian skin, transfersomes should be capable of passing through pores less than 50 nm in diameter under the influence of a suitable transdermal gradient (Cevc et al 1995). Only properly optimized and moderately-loaded carriers can pass through pores smaller than their own diameter. Increasing the concentration of membrane softening component beyond a certain level or even to the point of bilayer solubilization brings no advantages in terms of transcutaneous transpore efficiency. Only the optimum ratio of lipid and surfactant leads to flexibility of the transfersomal membrane (Hofer et al 2000). Lipid-to-surfactant ratio affects entrapment efficiency also and therefore this ratio was optimized by preparing formulations with different lipid-to-surfactant ratios. The optimum formulation was selected as the one that demonstrated good entrapment and a good elasticity value. Entrapment efficiency of transfersomes was determined by using Sephadex G-150 mini-column (Table 1).

Initially, increasing the concentration of sodium deoxycholate gave rise to a growth in vesicle size and as a consequence contributed to an increased entrapment value. The maximum entrapment efficiency was found to be 87.34 ± 3.81 with the formulation TD3 having a lipid-to-surfactant ratio of 85:15%, w/w. However, increasing the concentration of sodium deoxycholate could lead to pore formation in the bilayers, which resulted in decreased entrapment efficiency of the transfersomes.

Deformability index of transfersomes

The topical carrier system should be deformable so that it can pass easily through the minute pores present in the epidermis. Prepared formulations were subjected to a deformability study by extrusion measurement. The results were expressed in terms of deformability index (Table1).

Deformability is a unique characteristic of transfersomes. Deformability was found to increase as the concentration of sodium deoxycholate increased. Deformability was maximum (121.5 ± 4.21) with a soya phosphatidylcholine:sodium deoxycholate ratio of 85:15. Further increases in the concentration of sodium deoxycholate resulted in the lowering of deformability. Only the proper ratio of surfactant resulted in the maximum elasticity of the transfersomes bilayer. Although vesicles became larger in size, the amount of extruded material through 50-nm pores increased when sodium deoxycholate content was changed from 5 to 15% w/w. Any further increase in sodium deoxycholate content did not increase the amount of material extruded through the 50-nm pores, consequently there was a slight reduction in deformability. The formulation with the maximum entrapment and deformability was TD3. This was the formulation selected for further study.

In-vitro skin permeation experiment

The in-vitro skin permeation experiment used a locally fabricated Franz-diffusion cell. The experiment was carried out over 48 h and withdrawn samples were analysed using the BCA method. The antigen permeation pattern through excised rat skin is shown in Figure 1.

The results supported good permeation characteristics of the transfersomes. The percentage cumulative permeation of tetanus toxoid observed was 11.3 ± 0.66 and 17.6 ± 0.81, after 24 and 48 h, respectively, for tetanus toxoid-loaded transfersomes. Plain tetanus toxoid solution and the physical mixture of transfersomes and tetanus toxoid showed a significantly lower ($P < 0.05$) permeation profile as compared with tetanus toxoid-loaded transfersomes. Such a good performance of tetanus toxoid-loaded transfersomes was due to the good deformability of the vesicle to traverse the permeability barrier. The horny layer of the skin is associated with sparsely distributed, irregular pores referred to as real pores, which act as a permeability shunt. These virtual pores lower the skin permeability barrier and may contribute to transdermal flux (Paul & Cevc 1995). For the permeation of ultradeformable carriers (transfersomes), these pores are particularly useful and may be responsible for the initial enhanced permeation. The non-steady-state flux might have been due to the saturation of available opportunities in the form of virtual pores or channels in the skin. This requires further investigation.

Two types of interaction between the skin and vesicles might have effected the transdermal biomolecule delivery. Firstly, adsorption and fusion of biomolecule-loaded vesicles onto the surface of skin lead to a high thermodynamic activity gradient of the biomolecule–stratum corneum surface. Secondly, the effect of the vesicle on the stratum corneum causes a change in the bioactive permeation kinetics due to an impaired barrier function of the stratum corneum for the bioactive molecule (Touitou et al 1994; Fang et al 2001). The action of transfersomes as penetration enhancers may predominantly be on the intercellular lipid of the stratum corneum, raising the fluidity and weakness of the stratum corneum. The ultradeformable character of the transfersomes leads to their passage through the very fine

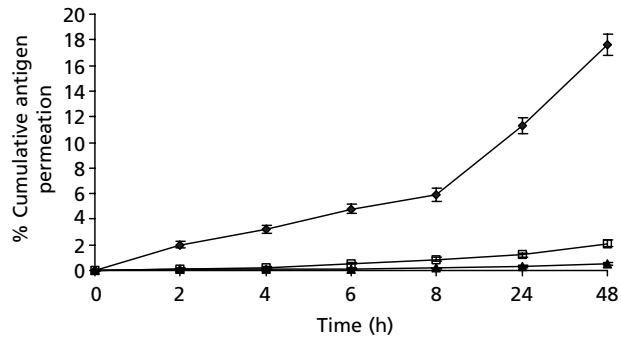

Figure 1 Percentage cumulative permeation through rat skin of tetanus toxoid entrapped in transfersomes (TD3) (●) vs plain tetanus toxoid (▲) and a physical mixture of transfersomes and tetanus toxoid (□).

pores in the skin under a suitable osmotic gradient. Phospholipids have a high affinity for biological membranes. Mixing the phospholipid of the carrier system with the skin lipid of the intercellular layers also contributes to the permeability of the skin to lipid vesicles (Weiner et al 1989; Ogiso et al 1997). Soya phosphatidylcholine contains phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and unsaturated fatty acid. The presence of unsaturated fatty acid may be responsible for enhanced permeation. The packing nature of unsaturated fatty acids changes the fluidity of the stratum corneum lipid structure and facilitates the permeation of the bioactive molecule (Valenta et al 2000).

Fluorescence microscopy

The penetration of transfersomes into intact rat skin was shown by fluorescence microscopy using 6-CF as a marker. Deep invaginations into the skin were observed (Figure 2a). Fluorescence microscopy indicated that the preferred route for transfersome penetration was between the cells in the corneocyte cluster. The distribution of fluorescence intensity at different depths in the skin demonstrated that the transfersome-associated dye was transported between and along the lipid stacks in the intercellular space. The fluorescence intensity gradually decreased with the depth in the skin. This may be attributed to the fact that transfersomes containing dye were facing sink conditions below the stratum corneum. As a result, dye dilution and finally its elimination through the lymphatic drainage system occurred (Cevc et al 1995). When the lipid suspension, the transfersomes, are placed on the skin and partially dehydrated by water loss due to evaporation, the transfersomes feel this gradient and try to avoid complete drying by moving along the gradient. The deformable nature of transfersomes allows them to pass through the narrow pores in the skin as revealed by dye penetration. Plain 6-CF solution was not found to penetrate into deeper skin layers; the fluorescence in this case was mainly confined to the superficial skin layers (Figure 2b).

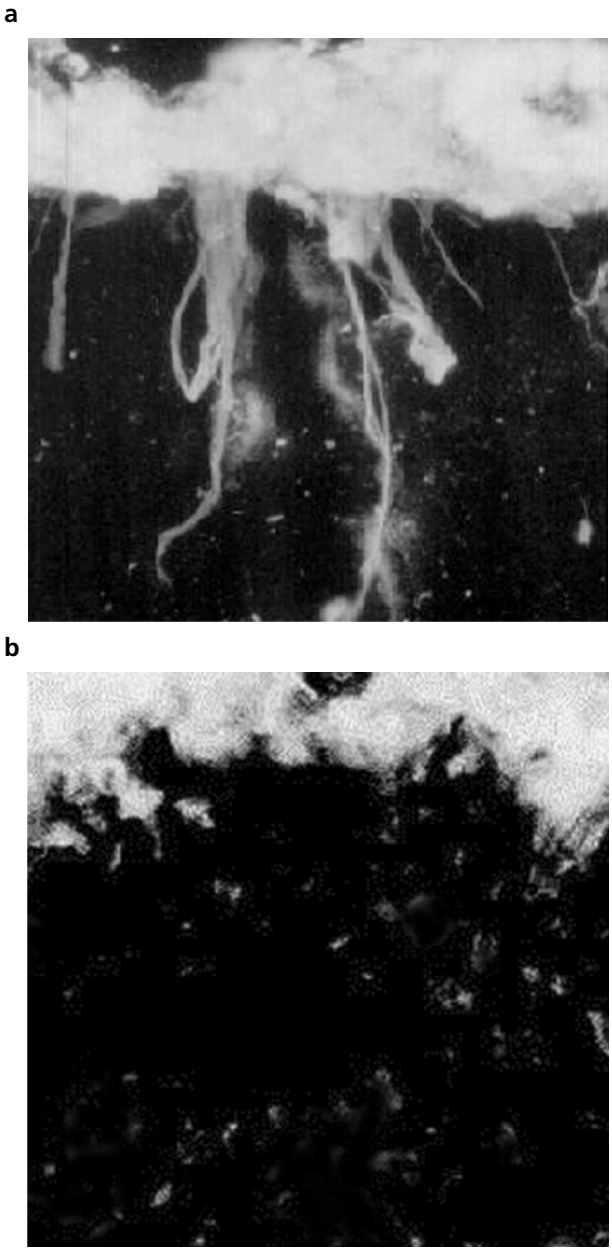

Figure 2 Fluorescence photomicrographs of the skin following application of (a) 6-carboxyfluorescein-loaded transfersomes and (b) plain 6-carboxyfluorescein solution.

Systemic IgG response

Topical immunization was carried out with tetanus-toxoid-loaded transfersomes, plain tetanus toxoid solution, and a physical mixture of tetanus toxoid and transfersomes. AATT was given intramuscularly. The systemic IgG response was measured on day 14, 28, 42, 56 and 90. Maximum response was observed after 42 days with AATT given intramuscularly followed by tetanus-toxoid-loaded transfersomes applied topically. AATT (intramuscularly) and tetanus toxoid-loaded transfersomes showed significantly higher ($P < 0.05$) immune responses compared with plain tetanus toxoid and the physical mixture of tetanus toxoid and transfersomes given topically. The physical mixture of tetanus toxoid and transfersomes showed a significantly ($P < 0.05$) higher immune response compared with plain tetanus toxoid, indicating the adjuvant nature of transfersomes (Figure 3). After secondary immunization on day 28, a significantly ($P < 0.05$) comparable immune response was observed with AATT given intramuscularly and tetanus toxoid-loaded transfersomes applied topically (Figure 4). High levels of antibody response after boosting indicated the presence of memory B and T cell populations evoked by primary immunization. After secondary immunization, the immune response was found to be sustained with a very gradual decrease in antibody titre. The results favour good immunoadjuvant action of transfersomes and reflect the potential for the topical delivery of the bioactive molecule (antigen).

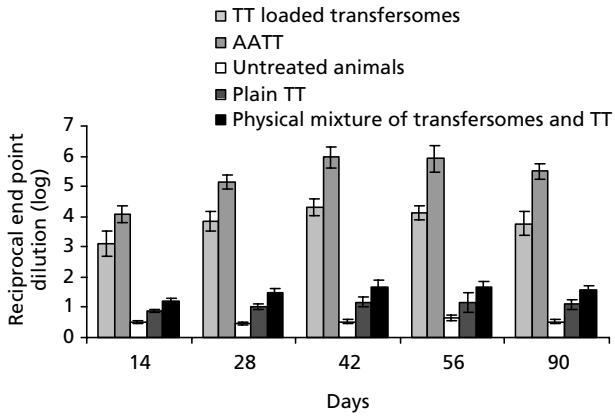

Figure 3 Induction of serum IgG response elicited following topical application of tetanus toxoid (TT)-loaded transfersomes TD3, plain tetanus toxoid solution or a physical mixture of transfersomes TD3 and tetanus toxoid. Immune response of AATT (intramuscularly) and untreated animals is shown also. (Protocol 1, no booster dose.)

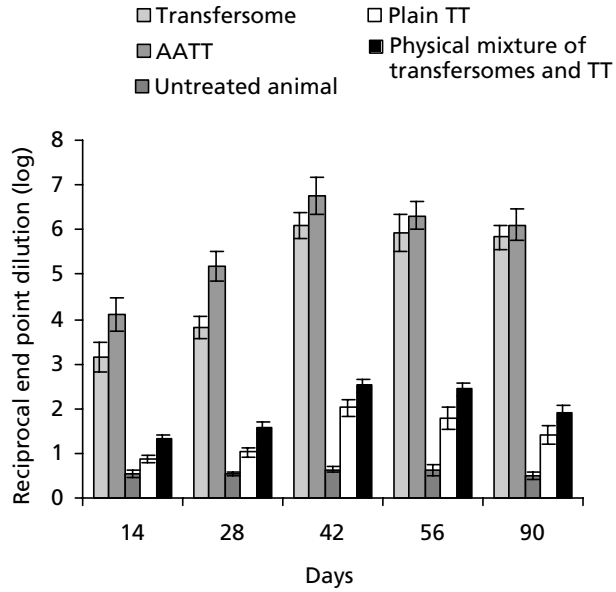

Figure 4 Induction of serum IgG response elicited following topical application of tetanus toxoid (TT)-loaded transfersomes TD3, plain tetanus toxoid solution or a physical mixture of transfersomes TD3 and tetanus toxoid. Immune response of AATT (intramuscularly) and untreated animals is shown also. (Protocol 2, booster dose was given on day 28.)

Antigens in conventional delivery systems are unable to penetrate through the intact skin. Classical penetration enhancers are also inefficient at eliminating this barrier. Thus we can infer that transfersome-mediated antigen delivery might not have been due to the penetration enhancement effect. Furthermore, the observed immune response might not have been due solely to better presentation of the antigen at the vesicle surface. A unique property associated with transfersomes is their deformability. This property in combination with sensitivity of transfersomes to the water gradient across the skin makes this carrier a potential system for topical immunization. The horny region of the skin is associated with sparsely distributed pores. These pores act as a permeability shunt and locally lower the skin permeability barrier (Paul & Cevc 1995). These pores are potential sites for deformable bodies, which are driven strongly by the transepidermal water gradient. Transfersomes are unique carriers having an ultradeformable nature and sensitivity to the transdermal water gradient. Deformability of the transfersomal membrane may be attributed to optimized membrane composition and manufacturing process.

The epidermal surface is known to be relatively dry, with a water content of less than 15% (Warner et al 1988). The local activity coefficient of water increases by at least 75% across the skin. Outer skin contains insufficient water concentration for most of the polar lipids (Cevc 1993). The lipid capability for spontaneous skin penetration via lipid vesicles results mainly from the transdermal osmotic gradient. Lipid hydrophilicity leads to xerophobia, the tendency to avoid dry surroundings and causes carriers sitting near or at the skin surface to resist dehydration to remain maximally swollen. Thus, transfersomes near the skin surface try to follow the local hydration gradient and thereby get into the deeper and better-hydrated skin strata. This causes the transfersome carrier to retract from the relatively dry skin surface and to get into the more humid region in deeper skin layers (Cevc & Blume 1992).

The immunity induced by topical immunization appears to be durable, as indicated by persistence of serum antibodies. The antibody response was almost similar in magnitude with that evoked by intramuscular immunization. This finding was consistent with studies using a patch containing heat-labile enterotoxin for transcutaneous immunization (Glenn et al 2000). Langerhans cells are the only antigen presenting cells in the un-inflamed epidermis (Udey 1997), therefore they play a vital role in the induction of the systemic immune response to topical immunization. Epidermal Langerhans cells form a semi-continuous network in the skin. The density of Langerhans cells in most areas of the skin is approximately 500–1000 cells mm^{-2} (Chen et al 1985; Bos et al 1987). They initiate, maintain and regulate adaptive immunities in the skin. These cells take up epicutaneous antigen, emigrate into the regional skin-draining lymph nodes and present the processed antigen to the T cells. Mature Langerhans cells express a high level of MHC (major histocompatibility complex) class I and class II antigens, co-stimulatory molecules and chemokine receptors. These are all important for the antigen presenting function of Langerhans cells (Cruz & Bergstresser 1990; Schuler & Steinman 1998). Langerhans cells present antigen to T cells in draining lymph nodes. They present antigen to naive T cells as well as to antigen specific T cells of $CD4^+$ and $CD8^+$ phenotype to stimulate both antibody and cellular immune responses. Thus Langerhans cells are pivotal for topical immunization and transfersomes are equally an important vehicle for non-invasive protein delivery to these immunocompetent cells.

Conclusion

This study favoured the deformable carrier (transfersomes) as a potential system for non-invasive antigen delivery via the skin. The response of transdermal immunization against tetanus toxoid using transfersomes was comparable with that achieved by intramuscular injection of the same dose of alum adsorbed tetanus toxoid. Transfersomes penetrated through the skin to deliver the antigen to the immunocompetent cells of the skin. This study revealed that transfersomes could be exploited as potential carriers for non-invasive topical immunization.

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