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Review Article

CLINICAL P TRANSFERSOME: A NEW TECHNIQUE FOR TRANSDERMAL DRUG DELIVERY

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ABSTRACT

A novel vesicular drug carrier system called transfersomes, which is composed of phospholipid, surfactant, and water for enhanced transdermal delivery. The transfersomal system was much more efficient at delivering a low and high molecular weight drug to the skin in terms of quantity and depth. The system can be characterized by in vitro for vesicle shape and size, entrapment efficiency, degree of deformability, number of vesicles per cubic mm. These carriers can transport pharmacological agents, including large polypeptides, through the permeability barriers, such as the intact skin. This capability depends on the self-regulating carrier deformability which exceeds that of the related but not optimized lipid aggregates by several orders of magnitude. Conventional lipid suspensions, such as standard liposomes or mixed lipid micelles, do not mediate a systemic biological effect upon epicutaneous applications. In contrast to this, the properly devised adaptable carriers, when administered on the intact skin, transport therapeutic amounts of biogenic molecules into the body.

Keywords: Transfersome, Transdermal delivery, transportation of biogenic molecules.

INTRODUCTION

Large biogenic or biotechnologic molecules are normally delivered into the body by means of an injection needle. However, numerous and ingenious attempts were made to improve on this[1,2]. They were based on inventive galenic formulations, including oral polymer[3], liposome[4] or microemulsion[5,6] suspensions, on the technical innovations, such as subcutaneous reservoirs [7] and pumps w8x, or on the unusual, e.g. rectal [9], periocular[10], intranasal[11] or dermal[6] applications. None of these approaches to date gave completely satisfactory results due to various reasons.

Adverse side effects, drug metabolism by first pass effect in the liver, poor patient compliance, or rejection of an invasive medication often hamper the success and efficacy of therapeutic treatment. To overcome these problems many drug carriers were developed such as Liposomes, dendrimers, and other complex polymers system.

Transport of the drug through skin is best route of drug delivery because of the skin is largest organ human organ with total weight 3 kg and a surface of 1.5 -2.0 m^2. Drug carries used in transdermal drug delivery such as liposomes, noisomes, or microemulsions has problem that they remains mostly confined to the skin surface and therefore do not transport drugs efficiently through the skin[12].

By using the concept of rational membrane design[13] we have recently devised special composite bodies, so-called Transfersomes[14], which overcome the filtration problem and

penetrate the skin barrier along the transcutaneous moisture gradient. Transfersomes are sufficiently flexible to pass even through the pores appreciably smaller than their own size. By optimally matching the penetrant adaptability to the transport-induced stress the size-exclusion principle is evaded nearly completely. The resulting highly deformable vesicles then pass through the narrow, otherwise confining, pores with the efficacy of water, 1000 times smaller. This leads the carrier through the "virtual" pores between the cells in the organ without affecting its biological and general barrier properties. Owing to this unusual barrier penetration mechanism, transfersome carriers can create a highly concentrated drug depot in skin[15], deliver material into deep subcutaneous tissue[16],or even deliver the drug into the systemic circulation[17].

Silent features of Transfersomes

- Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility.
- Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss.
- This high deformability gives better penetration of intact vesicles.
- They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin.
- They are biocompatible and biodegradable as they are made from natural phospholipids similar to liposomes.
- They have high entrapment efficiency, in case of lipophilic drug near to 90%.
- They protect the encapsulated drug from metabolic degradation.
- They act as depot, releasing their contents slowly and gradually.
- They can be used for both systemic as well as topical delivery of drug.
- Easy to scale up, as procedure is simple, do not involve lengthy procedure and unnecessary use or pharmaceutically unacceptable additives.

Limitations of transfersomes

- Transfersomes are chemically unstable because of their predisposition to oxidative degradation.
- Purity of natural phospholipids is another criteria militating against adoption of transfersomes as drug delivery vehicles.
- Transfersomes formulations are expensive.

Mechanism of Penetration of Transfersomes

Transfersomes when applied under suitable condition can transfer 0.1 mg of lipid per hour and cm2 area across the intact skin. This value is substantially higher than that which is typically driven by the transdermal concentration gradients. The reason for this high flux rate is naturally occurring "transdermal osmotic gradients" i.e. another much more prominent gradient is available across the skin [18]. This osmotic gradient is developed due to the skin penetration barrier, prevents water loss through the skin and maintains a water activity difference in the viable part of the epidermis (75% water content) and nearly completely dry stratum corneum, near to the skin surface (15% water content)[19].

This gradient is very stable because ambient air is a perfect sink for the water molecule even when the transdermal water loss is unphysiologically high. All polar lipids attract some water this is due to the energetically favorable interaction between the hydrophilic lipid residues and their proximal water. Most lipid bilayers thus spontaneously resist an induced dehydration [20,21]. Consequently all lipid vesicles made from the polar lipid vesicles move from the rather dry location to the sites with a sufficiently high water concentration [22, 23], So when lipid suspension (transfersomes) is placed on the skin surface, that is partly dehydrated by the water evaporation loss and then the lipid vesicles feel this "osmotic gradient" and try to escape complete drying by moving along this gradient[19]. They can only achieve this if they are sufficiently deformable to pass through the narrow pores in the skin, because transfersomes composed of surfactant have more suitable rheologic and hydration

properties than that responsible for their greater deformability[23] less deformable vesicles including standard liposomes are confined to the skin surface, where they dehydrate completely and fuse, so they have less penetration power than transfersomes. Transfersomes are optimized in this respect and thus attain maximum flexibility, so they can take full advantages of the trans-epidermal osmotic gradient (water concentration gradient)[24]. Transfersome vesicle can therefore adapt its shape to ambient easily and rapidly, by adjusting local concentration of each bilayer component to the local stress experienced by the bilayer as shown in fig 3[25].

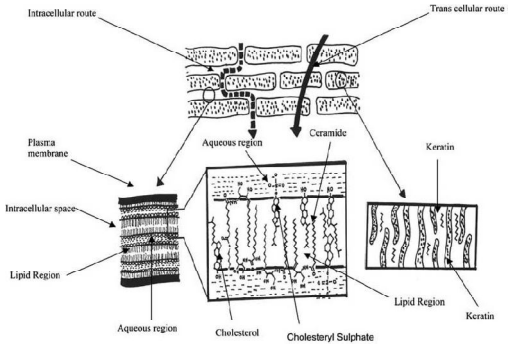

Fig. 1: Diagrammatic Representation of the Stratum Corneum and the Intercellular and Transcellular Routes of Penetration

Preparation of Transfersomes

Various published and patented procedure are available for the preparation of transfersome. Generally phosphatidylcholine is mixed in ethanol with sodium cholate or some other biocompatible surfactant. Subsequently a suitable buffer is added to yield a total lipid concentration of 10% w/w. the suspension is then sonicated, frozen, and thawed 2-3 times to catalyze vesicle growth and is finally brought to the preferred vesicle size by pressure homogenization, ultrasonication, or some other mechanical method. Final vesicle size, as determined dynamic light scattering, is approximately 120 nm for a typical transfersome preparation containing 8.7% by weight SPC, 1.3% by weight sodium cholate, and up to 8.5% by volume ethanol.

The best carrier composition has to be found experimentally and for each drug separately to obtain appropriate transfersome carriers with maximum deformability and stability[15].

Table 1: Different Additives Used in Formulation of Transfersome

Class	Example	Uses
Phospholipids	Soya phosphatidyl choline Dipalmitoyl phosphatidyl choline Distearoyl phoshatidyl choline	Vesicles forming component
Surfactant	Sod. cholate Sod.deoxycholate tween-80 Span-80	For providing flexibility
Alcohol	Sod. cholate Sod.deoxycholate tween-80 Span-80	As a solvent
Dye	Rhodamine-123 Rhodamine-DHPE Fluorescein-DHPE Nile-red	For CSLM study
Buffering agent	Saline phosphate buffer (pH 6.4)	As a hydrating medium

Characterization of Transfersomes

The characterization of transfersomes is generally similar to liposomes, noisomes and micelles.

1. **Entrapment Efficiency**

   The entrapment efficiency is expressed as the percentage entrapment of the drug added. Entrapment efficiency was determined by first separation of the unentrapped drug by use of mini-column centrifugation method. After centrifugation, the vesicles were disrupted using 0.1% Triton X-100 or 50% n-propanol. The entrapment efficiency is expressed as:

   Entrapment efficiency= (amount entrapped/ total amount added)*100.

2. **Vesicles Size and Size Distribution**

   The vesicles size and size distribution were determined by dynamic light scattering method (DLS), using a computerized inspection system (Malvern Zetamaster, ZEM 5002, Malvern, U.K.). For vesicles size measurement, vesicular suspension was mixed with the appropriate medium (7% v/v ethanol) and the measurements were conducted in triplicate[26].

3. **Degree of Deformability or Permeability Measurement**

   Degree of deformability is an important and unique parameter of transfersomal formulations because it

differentiates transfersomes from other vesicular carriers like liposomes that are unable to cross the stratum corneum intact. The deformability study is done against the pure water as standard. Transfersomes preparation is passed through a large number of pores of known size (through a sandwich of different microporous filters, with pore diameter between 50 nm and 400 nm, depending on the starting transfersomes suspension). Particle size and size distributions are noted after each pass by dynamic light scattering (DLS) measurements. The degree of deformability was calculated by using the following formula, as reported by Berge vanden et al. [27]:

$$D = J * \left(r_v / r_p \right)^2$$

where,

D = deformability of vesicle membrane

J = amount of suspension, which was extruded during 5 min

r_v = size of vesicles (after passes)

r_p = pore size of the barrier

4. Propensity of penetration

The magnitude of the transport driving force plays an important role:

Flow = Area x (Barrier) Permeability x (Trans-barrier) force

Therefore, the chemically driven lipid flow across the skin always decreases dramatically when lipid solution is replaced by the some amount of lipids in a suspension.

5. Confocal Scanning Laser Microscopy (CSLM) study

Conventional light microscopy and electron microscopy both face problem of fixation, sectioning and staining of the skin samples. Often the structures to be examined are actually incompatible with the corresponding processing techniques; these give rise to misinterpretation, but can be minimized by Confocal Scanning Laser Microscopy (CSLM). In this technique lipophilic fluorescence markers are incorporated into the transfersomes and the light emitted by these markers used for following purpose:

- For investigating the mechanism of penetration of transfersomes across the skin,
- For determining histological organization of the skin (epidermal columns, interdigitation), shapes and architecture of the skin penetration pathways. For comparison and differentiation of the mechanism of penetration of transfersomes with liposomes, niosomes and micelles.

Different fluorescence markers used in CSLM study are

- Fluorescein-DHPE(1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine-N-(5-fluores denthiocarbamoyl), triethylammonium salt)
- Rhodamine-DHPE (1,2-dihexadecanoyl-sn-glycero-3ogisogietgabikanube-N-Lissa mineTmrhodamine B sulfonyl), triethanolamine salt)
- NBD-PE (1, 2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-Benz-2- oxa-1, 3-diazol-4-yl) triethanolamine salt)
- Nile red

6. Number of Vesicles per Cubic mm

This is the most important parameter for optimizing the composition and other process variables. Transfersomal formulation (without sonication) was diluted five times with 0.9% of NaCl solution, and the number of transfersomes per cubic mm was counted by optical microscopy by using a haemocytometer. The transfersomes in 80 small squares were counted and calculated by using the following formula[27].

Total no. of transfersomes per cubic mm

$$= \frac{\text{Total number of transferosomes counted} \times \text{Dilution factor} \times 4000}{\text{Total number of squares counted}}$$

7. In vitro Drug Release

In vitro drug release study is performed for determining the permeation rate. Time needed to attain steady state permeation and the permeation flux at steady state and the information from in-vitro studies are used to optimize the formulation before more expensive in vivo studies are performed. For determining drug release, transfersomes suspension is incubated at 32°C and samples are taken at different times and the free drug is separated by mini column centrifugation [28]. The amount of drug released is then calculated indirectly from the amount of drug entrapped at zero times as the initial amount (100% entrapped and 0% released).

8. Stability Studies

Transfersomes stability was determined at 4°C and 37°C by TEM visualization and DLS size measurement at different time intervals (30, 45, and 60 days), following vesicles preparation.

CONCLUSION

Transfersomes are specially optimized particles or vesicles, which can respond to an external stress by rapid and energetically inexpensive, shape transformations. Such highly deformable particles can thus be used to bring drugs across the biological permeability barriers, such as skin. When tested in artificial systems. Transfersomes can pass through even tiny pores (100 mm) nearly as efficiently as water, which is 1500 times smaller. Drug laden transfersomes can carry unprecedented amount of drug per unit time across the skin (up to 100mg cm2h-1). The systemic drug availability thus mediated is frequently higher than, or at least approaches 80-90%. The bio-distribution of radioactively labeled phospholipids applied in the form of transfersomes after 24 hr is essentially the same after an epicutaneous application or subcutaneous injection of the preparations. When used under different application conditions, transfersomes can also positioned nearly exclusively and essentially quantitatively into the viable skin region.

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