IJPSR (2011), Vol. 2, Issue 4 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 December, 2010; received in revised form 02 January, 2011; accepted 02 March, 2011

TRANSFEROSOMES: AN EMERGING TOOL FOR TRANSDERMAL DRUG DELIVERY

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Keywords:

Transfersome,

Transdermal drug delivery,

Elastic vesicles,

Occlusion

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ABSTRACT

Transdermal drug delivery is proving to be superior to the conventional oral delivery of drugs owing to its distinct virtues. But it has got its own limitations as well- inability to transport large molecules, inability to overcome the barrier properties of stratum corneum and many more. Using this route of drug delivery along with novel approaches can help to solve these problems. Formulating the drug in a transfersome is one such approach. Transferosome is an ultradeformable vesicle, elastic in nature which can squeeze itself through a pore which is many times smaller than its size owing to its elasticity. Transferosomes are made up of a phospholipids component along with a surfactant mixture. The ratios of individual surfactants and total amount of surfactants control the flexibility of the vesicle. The uniqueness of this type of drug carrier system lies in the fact that it can accommodate hydrophilic, lipophilic as well as amphiphilic drugs. These drugs find place in different places in the elastic vesicle before they get delivered beneath the skin. Controlled release formulations can also be prepared with the help of transferosomes. Better or equivalent bioavailability has been reported in case of drugs like insulin, corticosteroids, etc. with the difficulty of administration by injection no more remaining. Peripheral drug targeting, transdermal immunization can also be achieved with this type of drug delivery system.

INTRODUCTION: Transdermal delivery of drugs through the skin to the systemic circulation provides a convinient route of administration for a variety of clinical indications. Transdermal delivery is gaining importance recently because of certain advantages over the conventional oral one [1, 2, 3]. The application of transdermal delivery to a wider range of drugs is limited due to the significant barrier to penetration across the skin which is associated primarily with outermost stratum corneum layer of epidermis [4, 5]. The skin structure looks as if stratum corneum cells are embedded in a pool of intercellular lipid lamellae [6]. These lamellae have a crucial role in imparting barrier properties to the stratum corneum [7, 8]. As a result, only milligram quantities of drug can be delivered by this route. This limits the application of this route to only potent drugs. Extensive work has been done in order to overcome the barrier properties of intact human skin. These include augmentation of skin permeability using penetration enhancers, use of forces which are not dependent on concentration gradient (iontophoresis, electroporation, phonophoresis, microneedles, jet injectors, etc.,) and many more. Transfersomes or other drug carrier systems like vesicles belong to the latter category.

Vesicles as drug delivery agents: Vesicular systems are gaining importance recently owing to their ability to act as a means of sustained release of drugs. These systems have several advantages: they can encapsulate both hydrophilic and lipophilic moieties, prolong half lives of drugs by increasing duration in systemic circulation due to encapsulation, ability to target organs for drug delivery, biodegradability, and lack of toxicity [9, 10, 11, 12, 13, 14, 15]. Vesicles have a unique structure which is capable of entrapping hydrophilic, lipophilic, amphiphilic and charged hydrophilic drugs. Vesicles are colloidal particles having a water filled core surrounded by a wall of lipids and surfactants (amphiphiles) arranges in bilayer. If the proportion of water is increased, these amphiphiles can form one or more concentric bilayers. Hydrophilic drugs find a place in the internal aqueous environment while amphiphilic, lipophilic drugs get entrapped in the bilayered wall with electrostatic and/or hydrophobic forces.

The flexible or deformable vesicles are called elastic vesicles or transferosomes. The concept and term of elastic vesicles was introduced first by by Gregor Cevc in 1991. Since then, huge amount of research is going on worldwide on these elastic vesicles under different titles like flexible vesicles, ethosome, etc. Transferosome is a term registered as a trademark by the German company IDEA AG, and used by it to refer to its proprietary drug delivery technology. The name means “carrying body”, and is derived from the Latin word 'transferre', meaning ‘to carry across’, and the Greek word ‘soma’, for a ‘body’. A Transferosome carrier is an artificial vesicle and resembles the natural cell vesicle. Thus it is suitable for targeted and controlled drug delivery. In functional terms, it may be described as lipid droplet of such deformability that permits its easy penetration through the pores much smaller than the droplets size. It is a highly adaptable and stress-responsive, complex aggregate.

When applied to the skin, the carrier searches and exploits hydrophilic pathways or 'pores' between the cells in the skin, which it opens wide enough to permit the entire vesicle to pass through together with its drug cargo, deforming itself extremely to accomplish this without losing its vesicular integrity. Interdependency of local composition and shape of the bilayer makes the vesicle both self-regulating and self-optimizing. This enables the Transferosome to cross various transport barriers efficiently. Transfersome penetrate the stratum corneum by either intracellular route or the transcellular route [16].

Liposomes and niosomes are the vesicular carrier systems which have received a lot of attention over the last decade as a means of transdermal drug delivery, in most cases transdermal drug penetration has not been achieved [17]. Thus, transferosomes are gaining importance these days. These vesicular transferosomes are several orders of magnitude more elastic than the standard liposomes and thus well suited for the skin penetration [18, 19].

Each vesicular carrier overcomes the skin barrier spontaneously, to deposit the drug into deep tissues, as it is drawn from the dry surface to the water-rich region beneath the skin. The carrier then avoids the local microvasculature in order to deposit the drug at various depths in or below the skin, where the active ingredient is preferentially and slowly released to its targeted tissue. The depth and extent of the drug deposition is chiefly controlled by the carrier composition and the Transferosome dose applied per unit area, rather than the total drug amount or concentration used. Transferosomes protects the encapsulated drug from metabolic degradation. They act as depot, releasing their content slowly and gradually [20].

Composition of Transferosomes: Transferosomes are composed of phospholipids like phosphatidyl choline which self assembles into lipid bilayer in aqueous environment and closes to form a vesicle. A bilayer softening component (such as a biocompatible surfactant or an amphiphile drug) is added to increase lipid bilayer flexibility and permeability. This second component is called as edge activator [18, 21, 22]. An edge activator consists usually of single chain surfactant that causes destabilization of the lipid bilayer thereby increasing its fluidity and elasticity.

The newer elastic vesicles were introduced by Van den berg in 1998, consisting of non ionic surfactant as the edge activator [23]. Flexibility of transferosomes membrane can be altered by mixing suitable surface active agents in the proper ratios. The resulting, flexibility and permeability optimized, Transferosome vesicle can therefore adapt its shape to surrounding stress easily and rapidly, by adjusting local concentration of each bilayer component to the local stress experienced by the bilayer. This flexibility also minimizes the risk of complete vesicle rupture in the skin and allows transferosomes to follow the natural water gradient across the epidermis, when applied under non occlusive condition.

TABLE 1: SHOWS THE COMPOSITION OF A TRANSFERSOME [24]

CLASS	EXAMPLE	USES
Phospholipids	Soya phosphatidyl choline, egg phosphatidyl choline, dipalmitoyl phosphatidyl choline	Vesicles forming complexes
Surfactant	Sod. cholate, Sod. deoxycholate, Tween-80, Span-80	For providing flexibility
Alcohol	Ethanol, methanol	As a solvent
Buffering agent	Saline phosphate buffer (pH 6.4)	as a hydrating medium
Dye	Rhodamine 123, Nile red	for confocal scanning Laser microscopy (CSLM)

Mechanism of Transport: The mechanism for penetration is the generation of “osmotic gradient” due to evaporation of water while applying the lipid suspension (transferosomes) on the skin surface. The transport of these elastic vesicles is thus independent of concentration. The trans-epidermal hydration provides the driving force for the transport of the vesicles [26]. As the vesicles are elastic, they can squeeze through the pores in stratum corneum (though these pores are less than

one-tenth of the diameter of vesicles) [27]. A Transfersome vesicle applied on an open biological surface, such as non-occluded skin, tends to penetrate its barrier and migrate into the water-rich deeper strata to secure its adequate hydration. During penetration through the stratum corneum, reversible deformation of the bilayer occurs. But it should be noted that while this deformation is occurring, vesicle integrity, gradient and barrier properties for the underlying hydration affinity should not be compromised.

Since it is too large to diffuse through the skin, the Transfersome needs to find and enforce its own route through the organ. The Transfersome vesicles usage in drug delivery consequently relies on the carrier's ability to widen and overcome the hydrophilic pores in the skin. Intracellular drug transportation may involve diffusion of vesicle lipid bilayer with the cell membrane like normal endocytosis. The mechanism is thus complex and involves advanced principles of elasto-mechanics combined with material transport and hydration/osmotic force.

Method of Preparation [27]: Phospholipids, surfactants and the drug are dissolved in alcohol. The organic solvent is then removed by rotary evaporation under reduced pressure at 40^{o}C. Final traces of solvent are removed under vacuum. The deposited lipid film is hydrated with the appropriate buffer by rotation at 60 rpm for 1 hour at room temperature. The resulting vesicles are swollen for 2 hours at room temperature. The multilamellar lipid vesicles (MLV) are then sonicated at room temperature. Sonication may be replaced by extrusion, low shear mixing (for formation of unilamellar vesicles) or high shear mixing (for formation of multilamellar vesicles.)

Characterization of Transferosomes [16, 20]: The mechanical properties and transport ability of a vesicle can be studied by measuring stress or deformation-dependent vesicle bilayer elasticity and permeability changes. The pressure dependent area density of the Transferosome suspension flux through a nano-porous filter can be determined for this purpose. For the proper Transferosome vesicles, "Penetrability" increases non-linearly (usually sigmoidally) with the flux driving force (head pressure).

Entrapment efficiency: Entrapment efficiency can be determined by separating the unentrapped drug. After centrifugation (to separate the unentrapped drug), the vesicle can be ruptured. Then appropriate analytical technique can be used to determine the amount of entrapped drug.

Vesicle diameter: Vesicle diameter can be determined using photon correlation spectroscopy or dynamic light scattering (DLS) method.

Confocal scanning laser microscopy (CSLM) study: This study enables comparison of transferosomes with liposomes, niosomes, etc. and study of mechanism of transfersome penetration. The principle is incorporation of a fluorescent marker which is lipophilic and which can emit light. This emitted light is used for further detection.

Degree of deformability or permeability measurement: The transfersome preparation is passed through many filters between pore sizes 50 to 400 nm. Vesicles retained on each filter are studied for particle size and distribution using dynamic light scattering technique. The degree of deformability can be determined using using the following formula,

$$D = J*(rv/rp)$$

Where, J- the amount of the suspension extruded during 5min.
rv - the size of the vesicle;
rp - pore size of the barrier.

***In vitro* drug release:** Transferosomes suspension is incubated at 32^{o}C using cellophane membrane. The samples are withdrawn at different intervals. Detection is done by various analytical techniques like U.V., HPLC, and HPTLC). Free drug is separated and its amount is calculated. From this, the drug release is calculated.

Vesicle shape and type: Transferosomes vesicles can be visualized by TEM, phase contrast microscopy, etc. The stability of vesicle can be determined by assessing the size and structure of vesicles over time. Mean size is measured by DLS and structural changes are observed by TEM.

Number of vesicle per cubic mm: Non-sonicated transfersome formulations are diluted five times with 0.9% sodium chloride solution. Haemocytometer and optical microscope can then be used for further study. The transferosomes in 80 small squares are counted and calculated using the following formula:

Total number of transferosomes per cubic mm = Total number of transferosomes counted * dilution factor* 4000

Penetration ability: Fluorescence microscopy is used to evaluate penetration ability of transferosomes.

Turbidity measurement: Turbidity of drug in aqueous solution can be measured using nephelometer.

Surface charge and charge density: Surface charge and charge density of transferosomes can be determined using zetasizer.

Drug Content: The drug content can be determined using a modified high performance liquid chromatography method (HPLC) method using a UV detector, column oven, auto sample, pump, and computerized analysis program.

Occlusion Effect: Occlusion of skin is considered to be helpful for permeation of drug in case of traditional topical preparations. But the same proves to be detrimental for elastic vesicles. Hydrotaxis (movement in the direction) of water is the major driving force for permeation of vesicles through the skin, from its relatively dry surface to water rich deeper regions [28]. Occlusion affects hydration forces as it prevents evaporation of water from skin.

Applications: Transferosomes as drug delivery systems have the potential for providing controlled release of the administered drug and increasing the stability of labile drugs. Very large molecules incapable of diffusing into skin as such can be transported across the skin with the help of transferosomes. For example, insulin, interferon can be delivered through mammalian skin. Delivery of insulin by transferosomes is the successful means of non invasive therapeutic use of such large molecular weight drugs on the skin. Insulin is generally administered by subcutaneous route that is inconvenient. Encapsulation of insulin into transferosomes (transfersulin) overcomes the problems of inconvenience, larger size (making it unsuitable for transdermal delivery using conventional method) along with showing 50% response as compared to subcutaneous injection [29].

Transferosomes have also been used as a carrier for interferons like leukocytic derived interferon-α (INF-α). Transferosomes have been widely used as a carrier for the transport of other proteins and peptides. Proteins and peptide are large biogenic molecules which are very difficult to transport into the body, when given orally they are completely degraded in the GI tract and transdermal delivery suffers because of their large size. Transferosomes help obtain some what similar bioavailability to subcutaneous injection. Human serum albumin or gap junction protein was

found to be effective in producing the immune response when delivered by transdermal route encapsulated in transferosomes [30, 31]. Transport of certain drug molecules that have physicochemical which otherwise prevent them from diffusing across stratum corneum can be transported.

Peripheral drug targeting: The ability of transferosomes to target peripheral subcutaneous tissues is due to minimum carrier associated drug clearance through blood vessels in the subcutaneous tissue. These blood vessels are non-fenestrated and also possess tight junctions between endothelial cells thus not allowing vesicles to enter directly into the blood stream. This automatically increases drug concentration locally along with the probability of drug to enter peripheral tissues.

Transdermal immunization: Since ultradeformable vesicles have the capability of delivering the large molecules, they can be used to deliver vaccines topically. Transferosomes containing proteins like integral membrane protein, human serum albumin, gap junction protein are used for this purpose. Advantages of this approach are injecting the protein can be avoided and higher IgA levels are attained. Transcutaneous hepatitis-B vaccine [32] has given good results. A 12 times higher AUC was obtained for zidovudine as compared to normal control administration. Selectivity in deposition in RES (which is the usual site for residence of HIV) was also increased [33].

NSAIDS are associated with number of GI side effects. These can be overcome by transdermal delivery using ultradeformable vesicles. Studies have been carried out on Diclofenac [34] and Ketotifen [35]. Ketoprofen in a *Transfersome* formulation gained marketing approval by the Swiss regulatory agency (SwissMedic) in 2007; the product is expected to be marketed under the trademark *Diractin*. Corticosteroids used to treat skin diseases are required at appreciably low doses than conventional formulations when formulated with transferosomes. Flexible vesicles of ethinylestradiol showed significant anti-ovulatory effects as compared to plain drug given orally and traditional liposomes given topically [36]**.** Transferosome based formulations of local anesthetics- lidocaine and tetracaine showed permeation equivalent to subcutaneous injections [37]. Anti cancer drugs like methotrexate were tried for transdermal delivery using transfersome technology. The results were favorable. This provided a new approach for treatment especially of skin cancer [38]. Extensive work has been done on other drugs like hormones and peptides viz Estradiol [39, 40], low molecular-weight Heparin [41], Retinol [42], Melatonin [43], etc.

CONCLUSION: The transdermal route of drug administration has been a route of choice since ancient times because of its merits. But it itself limits its use as it is unable to transport larger molecules, penetration through the stratum corneum is the rate limiting step, physicochemical properties of drugs hinder their own transport through skin. The development of novel approaches like transferosomes have immensely contributed in overcoming these problems. These elastic vesicles can squeeze themselves through skin pores many times smaller than their own size and can transport larger molecules.

This approach is safe in composition and hence safe to use with increased transdermal flux and improved site specificity. Drug release can be controlled whenever and wherever required. Interferons, proteins like insulin, NSAIDs, anti-cancer agents, anti- HIV agents have been successfully tested for delivery using this approach. Thus, this novel technique has got a great potential for overcoming current problems faced by the conventional techniques.

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