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Lipid Nanocarriers for Transdermal Anticancer Drug Delivery

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Abstract. Skin cancer is one of the most common malignant tumors worldwide but its incidence is continuously increasing. The conventional treatment methods are limited by the systemic toxicity, low bioavailability and insufficient targeting. Lipid nanocarriers can encapsulate a wide range of therapeutic agents and may help overcome the stratum corneum barrier, making them promising carriers for transdermal anticancer delivery. This review summarizes recent progress in lipid-based nanocarriers, including Liposomes, Ethosomes, Transfersomes and Transethosomes. The transdermal penetration mechanism, the preparation methods and the applications in delivery chemotherapeutic drugs, natural active ingredient and dual-drug combination therapy for skin cancer are focused on. Overall, the evidence reviewed here suggests that the design parameters of the carriers jointly determine the transdermal efficiency, the dual-drug co-loading strategy can achieve a synergistic anti-cancer effect and avoid multi-drug resistance. The transdermal mechanism is still unclear, and the key problems such as the process scale-up and the long-term safety still need to be tackled. This review provides a systematic framework for the understanding of the structure-activity relationship of the lipid nanocarrier and also gives a practical guidance for the rational design of next-generation transdermal delivery systems, and it is hoped that it can promote the clinical translation of these promising nanomedicines in the treatment of the skin cancer.

Keywords: Skin cancer, Lipid-based nanoparticles, Transdermal drug delivery, Nanomedicine

1. Introduction

Skin cancer is one of the most common malignancies in the world and its incidence continues to increase, so that it is nowadays a major public health problem. For example, epidemiological data indicate that more than one million of new cases of skin cancer are reported all over the world each year. Non-melanoma skin cancers (NMSCs), mainly basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are more common. It is well known that the main etiological factor is the Ultraviolet (UV) radiation is widely recognized as the principal environmental culprit, and its interaction with shifting lifestyle behaviors has further fueled the upward trend in skin cancer incidence [1].

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Current standard treatments for skin cancer include surgery, chemotherapy, radiotherapy, and immunotherapy. However, each of them has its own intrinsic disadvantages [1].

Transdermal drug delivery avoids the hepatic first-pass metabolism and the gastrointestinal degradation, allows for a local accumulation of the drug in the skin and reduces the systemic toxic side effects [2]. However, the brick-and-mortar architecture of the stratum corneum is a strong barrier that limits the percutaneous absorption of macromolecules and hydrophilic drugs [2, 3]. On the other hand, considering the systemic administration, drug concentrations at the target site may be low, necessitating repeated dosing, which can increase the burden on organs and reduce patient adherence.

The appearance of the nanotechnology offers some strategies to overcome this barrier. As one kind of nanocarrier, liposomes are among the most extensively investigated nanocarriers due to their phospholipid bilayer structure, the excellent biocompatibility, and biodegradability [4]. Liposomes can co-encapsulate the hydrophilic and hydrophobic drugs. They may also promote the skin penetration via the mechanisms of fusion with the SC lipids, the hydration induced swelling, and the intact vesicle translocation [2, 3]. Various deformable liposomal systems are developed to enhance the transdermal efficacy. Ethosomes, which contain a high concentration of ethanol, can increase the deformability of the vesicle and reduce the phase transition temperature of the SC lipids, so that it can significantly enhance the depth of the drug penetration into the skin [5, 6]. In transfersomes, edge activators are incorporated into the vesicles to enhance their elasticity, and it can traverse the skin pores smaller than their own size under the driving force of the transdermal hydration gradients, thus they can achieve the drug delivery [7, 8]. As the second generation of the lipid nanoparticles, nanostructured lipid carriers (NLCs) form the incomplete crystalline lattices by the blending of the solid and liquid lipids, which improves the drug loading capacity and the colloidal stability. Besides, NLCs can form the occlusive films on the skin surface to reduce the transepidermal water loss (TEWL) and enhance the drug permeation [3, 7]. The strategy of the dual-drug co-delivery liposome which encapsulates two synergistical agents in one carrier can provide the multi-targeted intervention and avoid the MDR [4].

Although significant progresses have been achieved, transdermal delivery using lipid based nanocarriers still suffers from some problems, such as poorly elucidated permeation mechanisms, undefined impact of some critical formulation parameters, problems in scaling up manufacturing and last but not least, concerns about long-term safety. For this reason, a systematic synthesis of what is known nowadays about their transdermal mechanisms, of their carrier design principles, and of their progress in clinical translation is of paramount importance for the development of basic research and for the clinical application of this field.

This review examines lipid-based nanocarriers for transdermal anticancer delivery. It includes permeation mechanisms, use in chemotherapeutics, bioactives, nucleic acids, and immunotherapeutics as well as in co-delivery. It also analyses the main determinants of the efficiency and the problems for clinical translation in order to point future directions for skin cancer therapies.

2. Structure of the skin barrier and the basic principle of transdermal drug delivery

2.1. Anatomical structure of the skin and the "brick-mortar" model of the stratum corneum

The skin is the largest organ of the human body and covers an area of about 1.5-2.0 m^2. It consists of three main layers, the epidermis, dermis and the subcutaneous tissue. The outermost layer of the epidermis, the stratum corneum (SC) is the barrier to the transdermal drug absorption [9]. The SC is

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composed of 10-15 layers of flat, anucleate keratinocytes, that are embedded within a highly ordered intercellular lipid matrix [9]. This is classically described as a "brick-and-mortar" where corneocytes act as the "bricks" that are mostly made of keratin, and the intercellular lipids form the "mortar"[1, 2]. This type of structure gives the skin an exceptional barrier function, preventing the penetration of the external substances and water loss, but it is also the central problem of the transdermal drug delivery [3, 9].

2.2. Main pathways of drug percutaneous absorption

There are three main pathways for drugs to penetrate the stratum corneum and enter the viable epidermis and dermis [9]: transcellular pathway in which drugs pass sequentially through keratinocytes and their intercellular lipid matrix, and therefore, need to have multiple partitioning between the hydrophilic and the lipophilic environment. Because of the tortuous route, this pathway usually contributes little [2]. Intercellular pathway, in which the drugs diffuse along the continuous lipid matrix between the keratinocytes, which is the route of passive transdermal absorption of most of the drugs, and the resistance is mainly from the highly ordered arrangement of the lipids [2, 9]. Follicular pathway which the drug can bypass through the stratum corneum barrier directly, via the skin appendages such as hair follicle and sweat glands. Although these appendages account for only about 0.1% of the total skin surface area, they may play a big role in the transdermal absorption of the ions and large-molecule drugs [4].

2.3. Physicochemical factors influencing the transdermal absorption of drugs

The effectiveness of the transdermal absorption is ruled by the drug physicochemical properties and the compatibility with the stratum corneum lipids [2]. Molecules with a molecular weight below 500 Da generally permeate more readily because they encounter less steric hindrance [4]. A log P value between 1 and 3 may favor partitioning into the lipid matrix and subsequent release into the aqueous epidermis [2]. The lower is the melting point the higher the thermodynamic activity and the diffusion [9, 10]. The pKa and ionization state are also important. The non-ionized form may permeate more readily because it is more lipophilic, whereas the ionization reduce the flux. Thus the drug pK a relative to the pH of the skin determine the permeation potential [4]. Most of the anticancer agent with high MW, unfavorable log P, or with an extensive ionization, do not pass by a passive delivery [4, 9].

3. Types, preparation and characterization of lipid nanocarriers

3.1. Liposomes, ethosomes, transfersomes and transethosomes

Liposomes are made of phospholipid bilayers and can encapsulate both the hydrophilic and lipophilic drugs [4]. The preparation methods of the conventional liposomes mainly include the film hydration method, reverse-phase evaporation, and ethanol injection [2, 4]. The most classical method is the film hydration method. Phospholipids and cholesterol are dissolved in some organic solvents, then they are evaporated under reduced pressure, a thin film is formed then hydrated with aqueous phase to get the multilamellar vesicles. The size can be further reduced to 100-200 nm by extrusion or sonication [4]. Because their structure is similar to the cell membrane, the conventional liposomes have good biocompatibility. But they are a little bit stiff and are not able to penetrate the

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narrow intercellular spaces of the stratum corneum, so they are only in the hair follicle or on the skin surface [2].

Ethosomes are flexible vesicles obtained by addition of high concentration of ethanol in the liposome formulation [2, 5, 6]. They can be prepared by cold or by hot method: in the first one the slow addition of the aqueous phase to the phospholipids dissolved in ethanol; in the second one the phospholipids are hydrated at 40 °C and then mixed with the ethanol [5]. The presence of ethanol gives the negative charge and the very good deformability of the vesicles. The ethanol is a penetration enhancer which fluidizes the stratum corneum lipids and increases the drug permeation depth in the skin [6, 8]. Studies have reported that ethosomes can deliver drugs into deeper skin layers, including the dermis, and can increase transdermal flux relative to conventional liposomes [2, 5].

Transfersomes are vesicles in which the edge activators are introduced into the phospholipid bilayer of the vesicles. These agents disorder the regular arrangement of the lipid bilayer and greatly increase the flexibility and deformability of the vesicle [6, 11, 12]. The film hydration method is still the most used preparation technique [11]. Transfersomes can at least in principle traverse the skin pores several times smaller than the diameter of the vesicles, driven by the transdermal hydration gradients, and have the much superior transdermal efficiency compare to the conventional liposomes [8, 12].

Transethosomes contain both ethanol and edge activators, and combine the structural features of both ethosomes and transfersomes. Transethosomes are the development direction of third-generation flexible lipid vesicles [7, 11]. The deformability of transethosomes is mainly from the effects of ethanol and edge activators synergic [6, 12]. The differences among the three types of flexible lipid vesicles are listed in Sheet 1.

Table 1. Composition and function of three types of flexible lipid vesicles

Carrier type	Component	Deformation source	Driving force	Advantage
Ethosomes	Phospholipid, Ethanol	Ethanol fluidized lipid bilayer	Concentration gradient, Ethanol promotion	Simple preparation
Transfersomes	Phospholipids, Marginal activator	Disrupte order of lipid	Hydration gradient	High transdermal flux
Transethosomes	Phospholipids, Ethanol, Peripheral activator	Synergistic effect of ethanol and edge activators	Concentration gradient, Hydration gradient	Most deformable

3.2. Nanostructured lipid carriers

Nanostructured lipid carriers (NLCs) are the second generation of lipid nanoparticles. The only difference is the composition of the lipid matrix; a mixture of solid and liquid lipids in certain proportions form an incomplete crystalline lattice structure [1, 7]. NLCs are prepared either by high-shear homogenization or microemulsion method. The particle size is in the range of 100-300 nm, and zeta potential is usually between -20 and -60 mV. The encapsulation efficiencies are over 80% [7, 10]. In transdermal applications, NLCs form occlusive lipid film on the surface of the skin, which reduces transepidermal water loss (TEWL), thus the stratum corneum is more hydrated and the drug is more penetrated [1, 9]. The first generation of solid lipid nanoparticles (SLNs) suffer from low drug loading capacity and drug leakage during storage, due to crystallization of the lipids [7].

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In their review, Mall systematically summarized the application of NLCs in the treatment of skin cancer. They pointed that NLC formulations have superior permeation performance and stronger cytotoxicity compared with traditional formulations [7]. Also, in their review, Amparo pointed out that NLCs as one type of nanocarrier for local cancer treatment, have good application prospects in local drug administration for skin cancer such as melanoma [1]. Moreover, NLCs can also avoid first-pass metabolism through lymph absorption, so more drugs can go to the target site, drug release time can be prolonged and the patient compliance can be improved [7]. The amount of drug retained in the epidermis by NLCs can be 3 to 5 times higher than that of conventional formulations [10].

3.3. Physicochemical characterization parameters of lipid nanocarriers

Four parameters are used to evaluate the quality of the lipid-based nanocarriers: particle size, polydispersity index (PDI), zeta potential, and encapsulation efficiency [4, 9]. Particle size is measured by dynamic light scattering (DLS). Particle size below 300 nm is required to make sure that the vesicles can penetrate the intercellular channels of the stratum corneum; the smaller the particle, the larger the surface of the particle so that more contact with the skin is possible [2, 4]. PDI is the homogeneity of the particle size distribution. A value of 0.3 or lower generally indicates a relatively uniform size distribution, which is good for batch to batch reproducibility [4]. Zeta potential shows the surface charge of particles and the electrostatic stability of the system; the |zeta potential| > 30 mV is in general enough to ensure the system stability and to reduce aggregation [2, 4]. The encapsulation efficiency is the proportion of the drug that is successfully loaded into the carrier and it is calculated as:

$$encapsulation\ efficiency = \frac{total\ drug\ amount - free\ drug\ amount}{total\ drug\ amount} \quad (1)$$

The level of encapsulation efficiency is the one that determines the drug loading capacity per unit dose. Because of their biocompatibility, biodegradability, and similarity with the structure of natural epidermal lipids, lipid nanocarriers can act at the molecular level with the stratum corneum lipid membrane and thus, allow the skin penetration [2, 9].

4. Transdermal penetration mechanism of lipid nanocarriers

4.1. Fusion mechanism between liposomes and the stratum corneum lipids

The phospholipid bilayer of liposomes is structurally similar to the lipids between the keratinocyte cells, so that they can make the molecular level interaction after the local application [2, 9]. The drugs that they encapsulate can be released into the lipid continuous phase by the liposomes directly fusing with the lipid lamellae of the keratinocyte layer. This happens in a way that, since phospholipids have a thermodynamic tendency to integrate into the given existing lipid matrix, the fusion of liposomes with the lipid lamellae of the keratinocyte layer will temporally break the highly ordered arrangement of the keratinocyte lipids arrangement and make a way for the drugs to diffuse [2, 9].

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4.2. Hydration and keratinization layer expansion

Lipid nanocarriers, in particular nanostructured lipid carriers (NLCs), are able to form occlusive lipid membrane on the skin surface. The occlusion effect lowers the transepidermal water loss (TEWL) and raise the water content in the stratum corneum [7, 9]. The trans-epidermal hydration gradient not only helps the penetration of hydrophilic drugs by the aqueous channels, but also induces the expansion of the keratinocytes and loosens the dense "brick-mortar" structure, and decreases the diffusion resistance of the lipid matrix between cells [2, 7, 9].

4.3. Deformation and passage mechanism of flexible liposomes

Transfersomes and transethosomes have the excellent deformability. That is, they can pass through the pores of the skin that are much smaller than their own diameters [6, 11, 12]. The deformation is induced by the so called transdermal hydration gradient, that is, the physiological driving force by the difference of moisture between the quite dry part of the keratin and the hydrated part of the active epidermis [6]. Under this driving gradient, the flexible vesicles are pulled through the intercellular channels in their intact form and the encapsulated drugs are delivered to the deeper layers of the skin without compromising the integrity of the vesicles [12].

4.4. Fluidizing effect of ethanol on the lipids of the stratum corneum

The ethanol in the liposomes and lipid nanoparticles has two functions [5, 6]. If the liposomes and lipid nanoparticles are applied locally, the ethanol will insert into the polar head regions of the lipid components of the stratum corneum and disrupts the lateral packing, cholesterol and free fatty acids, and lowers the phase transition temperature of the lipid bilayer, the stratum corneum becomes more fluid and permeable [5, 8]. On the other hand, the ethanol will make the vesicle membrane itself to be fluidized, and enhance the deformability of the vesicles [6]. The fluidization effect at the barrier layer and the carrier layer at the same time is the reason why the vesicles with ethanol is better than the traditional liposomes in terms of the penetration depth [5, 6].

4.5. Edge activators enhance the elasticity of vesicles

Edge activators are single-chain surfactants which are placed in transferosomes and transethosomes [6, 11, 12]. Because of the large curvature radius of these molecules, they can break the regular disposition of the phosphatidyl chains in the bilayer and to create local defects of the membrane that give the membrane high flexibility [11]. Thus, the elasticity of the vesicles is so high, that they can be deformed a lot by the external forces and to push through the narrow intercellular channels [12]. While in the case of ethanol this action is mainly on the stratum corneum, in the case of edge activators we have the direct action on the carrier membrane, so that the vesicles will be always intact and work during the difficult penetration through the stratum corneum barrier [6, 11].

5. Applications of lipid nanocarriers in transdermal delivery of anti-tumor drugs

5.1. Transdermal delivery of small moleculer chemotherapy drugs

Lipid nanocarriers have been extensively studied for the transdermal delivery of small-molecule chemotherapeutic drugs [8]. 5-Fluorouracil, as a hydrophilic drug, has poor skin permeability. However, when delivered through ultradeformable liposomes, especially when co-loaded with

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resveratrol, its transdermal flux and skin deposition significantly increase [8]. Doxorubicin, despite its high molecular weight and hydrophilicity, has been successfully delivered through delivery vehicles and liposomes; studies have shown that when doxorubicin is co-loaded with ceramide or hispolon in liposomes, it exhibits higher cellular uptake and cytotoxicity in B16BL6 melanoma cells [8]. Paclitaxel, as a highly lipophilic drug, has been formulated into delivery vehicles and achieved enhanced transdermal flux and excellent tumor suppression effects in melanoma models [6]. Mitoxantrone, after delivery through liposomes, has significantly superior in vitro permeability and in vivo anti-melanoma efficacy compared to free drug solutions [8].

5.2. Transdermal delivery of natural active ingredients

Natural bioactive compounds have received extensive attention in the treatment of skin cancer due to their excellent safety and multi-target anti-cancer activity [8]. Curcumin, as a lipophilic polyphenol, has poor water solubility and low skin permeability. It has achieved a synergistic anti-tumor effect in skin cancer treatment through co-delivery with STAT3 siRNA via cationic liposomes [8]. Resveratrol and quercetin are two polyphenols with synergistic anti-cancer and antioxidant activities. They have been co-loaded into liposomes and demonstrated excellent tissue repair and anti-inflammatory effects in a TPA-induced mouse model of skin inflammation, with almost complete retention of DPPH free radical scavenging ability [8]. After delivery via liposomes, sulforaphane showed significantly enhanced in vitro transdermal permeability and anti-cancer activity against SK-MEL-28 melanoma cells [5].

5.3. Dual-drug co-loaded liposome synergistic therapy strategy

Dual-drug co-loaded liposomes represent an advanced strategy for achieving synergistic anti-cancer effects while avoiding multidrug resistance [4, 8]. Doxorubicin and celecoxib are co-loaded into liposomes and combined with microneedle pretreatment, showing a significantly enhanced tumor suppression effect and extremely low systemic toxicity in the B16 melanoma mouse model [8]. The liposome system co-loading doxorubicin and C8-squalamine has the highest cellular uptake and cytotoxicity in B16BL6 cells [8]. The 5-fluorouracil and cetuximab immunoliposome system demonstrates the potential of combining chemotherapy drugs with EGFR-targeting antibodies for the treatment of squamous cell carcinoma, with a significant reduction in tumor volume after iontophoretic [8]. Curcumin and STAT3 siRNA are co-loaded into deformed cationic liposomes, which can effectively inhibit STAT3 protein expression and tumor growth after iontophoretic [8]. Additionally, the liposome system co-loading quercetin and resveratrol significantly inhibits MPO activity and edema in the mouse model [8]. These studies indicate that dual-drug co-loading in liposomes can achieve multi-target intervention, reduce drug resistance, and achieve better therapeutic efficacy than single-drug treatment [4, 8].

6. Factors affecting the transdermal efficiency of lipid nanocarriers

6.1. Particle size and surface charge of the carrier

Particle size is one of the important factors which determine the transdermal efficiency of lipid nanocarriers [2, 4]. Usually, the particle size should be below 300 nm, in order to penetrate the intercellular channels of the stratum corneum; the smaller the particle size, the greater the specific surface area and the closer the contact with the skin [4, 9]. Vesicles smaller than 50 nm may be

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rapidly cleared before being delivered to the deeper layers [4]. Surface charge also affects the interaction between vesicles and the negatively charged stratum corneum lipid matrix [4, 9]. The absolute value of Zeta potential above 30 mV is in general considered to assure the stability of the dispersion and the prevention of aggregation [2, 4].

6.2. Lipid composition and membrane fluidity

The lipid composition of the lipid nanocarriers directly determines their membrane fluidity, deformability and interaction with the stratum corneum lipids [2, 5, 9]. The addition of the ethanol in the liposomes and in the lipid carriers reduces the phase transition temperature of the bilayer, so that the membrane is more fluid and deformable [5, 9]. The incorporation of the edge activators in the carrier also disrupts the regular lipid packing, so that the elasticity of the vesicle is enhanced [6, 11]. The cholesterol content regulates the rigidity of the membrane and the drug retention capacity: a higher cholesterol content can improve the drug loading and the sustained release, but it can also impair the deformability required for a deep skin penetration [4]. The choice of the phospholipids also affects the performance of the carrier: the unsaturated phospholipids give higher fluidity but are more prone to oxidation, while the saturated phospholipids have higher stability but lower flexibility [2, 9].

6.3. Physicochemical properties of the drug itself

The inherent physical and chemical properties of the delivered drugs affect their transdermal delivery efficiency [4, 9]. The drugs with molecular weight lower than 500 Da, suitable lipophilicity and low melting point are usually easier to achieve passive transdermal delivery [4, 9]. For the drugs which are deviated from these drugs characteristics, the encapsulation efficiency in lipid carriers becomes important [4]. The pKa and ionization state of the drug affect its distribution between the carrier and the stratum corneum lipids, and the non-ionized form is usually more prone to penetration [4, 9]. The solubility of the drug in the lipid matrix determine its drug loading and release kinetics, and high solubility usually leads to a higher encapsulation rate and more sustained release [9]. These drug-related factors emphasize the need to customize the carrier design strategies for each specific physical and chemical characteristic of each anti-cancer drug [4, 9].

7. Conclusion

This article systematically reviews the application of lipid nanocarriers in the context of skin cancer treatment for the transdermal delivery of anti-tumor drugs. The skin barrier, especially the keratin layer with its unique "brick-and-mortar" structure, limits the passive penetration of most of the chemotherapy drugs. The application of lipid nanocarriers provides in turn, diverse solutions to this problem by various penetration-promoting mechanisms. The unique structural and functional characteristics of each of the carrier types, allow for the development of custom delivery strategies for drugs with different physical and chemical properties. The application of dual-drug co-loading systems also suggests that this approach may achieve the synergistic anti-cancer effect and avoid the multi-drug resistance.

In this article, we have systematically clarified the relationship between the carrier design parameters and the transdermal delivery efficiency, and we have outlined design principles for nanocarrier formulations. The finding of specific carrier mechanisms for skin barrier overcoming provides practical guidance for the next generation delivery. The reported validation of the dual-drug

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co-loading strategy also shows the clinical potential of the combined treatment regimens of multiple oncogenic pathways.

It should be noted that most of the studies that we have summarised in this section are still at the preclinical stage, and have not been explored in terms of long term safety, the feasibility of large-scale production or route(s) of regulatory approval. Converting these interesting laboratory findings in clinically feasible products will still need to be assessed in human subjects under real-world conditions.

Future work should focus on the development of intelligent responsive-release systems that will react to some particular trigger in the tumor microenvironment. Using artificial intelligence to design the formulation can speed up the determination of the best carrier composition for some particular drug-carrier combination. Moreover, the development of scalable production technologies and the setting up of some standard QA protocols are essential to bridge the gap between the research of new solutions and the clinical use of more safer and more effective treatments for skin cancer treatment.

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