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SELECTIVE DRUG DELIVERY THROUGH AND WITHIN SKIN USING LIPOSOMES

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THE topical route of administration has been utilised either to produce local effects for treating skin disorders, or to produce systemic drug effects. In the first case, the penetration of the drug into the skin and its subsequent localization at the site of action is desirable. On the other hand for systemic effect, the drug should penetrate through the skin and reach the blood circulation in order to provide a therapeutically effective drug concentration. The principal factors involved in the localization and/or cutaneous or percutaneous absorption of substances are the properties of[1].

(a) the drug,
(b) the tissues (i.e. the skin) of the site of application and
(c) the vehicle.

In formulation of topical dosage forms the use of various new vehicles containing penetration promoters or drug carriers to ensure adequate penetration and/or localization of the drug have been attempted to achieve local effects or to ensure adequate percutaneous absorption to produce a desired systemic effect.

CUTANEOUS AND PERCUTANEOUS ABSORPTION

The main problem in dermatopharmacotherapy is the penetration of skin by most of drugs as with only a small portion of dose finally reaches the site of action within the skin, producing limited local activity. Moreover, a few drugs which penetrate the skin easily are quickly removed by blood circulation, thus producing systemic rather than local effect[2]. The best avenue to improve drug penetration and/or localization is obviously to manipulate the vehicle or to utilise a drug carrier concept.

One of the most debated area of skin research is the mode of penetration of various substances into or through skin[3-5]. The permeability and barrier function of skin can be attributed to basal layer, the stratum compactum and the stratum corneum[6-8]. The most recently acceptable theory assumes that polar and nonpolar lipids arranged in lamellar bilayers in stratum corneum interstices are responsible for barrier function[9-12]. Different routes for transport of molecules through the skin have been proposed[13,14]. The main pathway for penetration are considered to be the epidermis, either through the cells or between them; another pathway through the hair follicles or the sweat or sebaceous glands. The transfollicular pathway might be an important shunt[15].

DERMAL DRUG DELIVERY

In treating skin diseases the primary purpose of applying drugs to the skin is to induce local effects at a very close to the site of application. In such cases cutaneous absorption is desirable, but percutaneous absorption is not. In the case of dermatopharmacotherapy, the realization is to develop a selective delivery system that enhances penetration of active ingredient into the skin, localizes the drug at the site of action and reduces the per-

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cutaneous absorption. In such cases, a special vehicle or more often, a drug carrier controls or influences the pharmacokinetic fate of the active ingredient. Among a variety of drug carriers, liposomes seem to have the best potential[16].

LIPOSOMES AS DRUG CARRIER

Extensive literature exists on liposomes describing them as drug carriers[17,18]. In addition liposomes can act as slow release vehicles[19,20]. Several reports indicate that liposomes, depending on their nature (composition, size, surface charge), the drug and other ingredients present in liposomal drug product can fulfil both the requirements of system for localized drug effect as well as of drug carrier[16,20-30].

The major components of liposomes are lipids (mainly phospholipids and cholesterol), water, drug, electrolytes and possibly antioxidants, preservatives and viscosity inducing agents. Hydrogenated soya lecithin provided best results in terms of biocompatibility[31]. Since liposomes are made up of substances similar to cell membranes, it is assumed that they are biocompatible and biodegradable preparations. Owing to their biphasic nature, both lipophilic and hydrophilic ingredients can be accommodated in the liposomes. Cholesterol is added to stabilise the liposomal membrane and to minimise the leaching out of water-soluble drug. Electrolytes are used to enhance the lipid bilayer formation and to provide isotonicity.

In the case of topically applied liposomes it is desirable to use viscosity inducing agents to produce a consistency that makes the product easy to apply, give it good cosmetic properties and gains patient acceptability. Other auxiliary agents such as antioxidants and preservatives could also be included.

RATIONALE AND ADVANTAGES OF CONTROLLED DRUG DELIVERY WITH LIPOSOMES

The rationale of encapsulating a drug within liposomes is to prevent its metabolism and rapid removal by the blood circulation after its topical administration, so that the drug forms a depot[19,20].

The liposomes are ideally suited for drug delivery by virtue of following properties.

- ★ They can accommodate both water and oil-soluble compounds
- ★ They are biocompatible and biodegradable
- ★ They protect the encapsulated drug from metabolic degradation
- ★ They act as depot, releasing their contents slowly and gradually

Liposomes can be used to

- — Target or transport the drug within the body to the site of action
- — Localize the drug when the site of action is close to the site of administration
- — To act as slow release vehicles
- — Enhance percutaneous absorption
- — Enhance cutaneous absorption and decrease drug clearance from the dermis
- — Prevent or minimize drug absorption into and through the skin

SELECTIVE DRUG DELIVERY THROUGH AND WITHIN SKIN USING LIPOSOMES

The most intensive field of liposome research is related to the development of a selective drug delivery system suitable for cancer chemotherapy. The latest effort in this field is to test the immunospecific targeting of liposomes with the aid of antibodies[32-34]. When liposomes are injected intravenously, passive targeting can be achieved, because they are 'filtered out' by the reticuloendothelial system. Consequently, liposomes can serve as efficient delivery system for targeting of drugs to RES[35]. A successful targeting by liposomes can be achieved with immunomodulators such as muramyl di- or tripeptides[36]. In most of cases, liposomes have been investigated for systemic drug delivery; only a few studies have been devoted on their potential for topical application of drug[27-29].

Liposomes were found to be suitable for localization of topically applied drug at or near the site of application. The localizing effect is mainly due to the fact that liposomes especially MLV types, may act as slow release vehicles. There are indication the most liposomal formulations in multiple dose topical treatment provide higher drug concentrations in the skin than conventional dosage forms[16,21,22,24,27,39,40,42,44]. Certain liposomal formulations greatly enhance both dermal and transdermal drug delivery[16,17,37,38]. With appropriate formulation, the drug could be targeted even within the skin[16,20].

LIPOSOME SKIN INTERACTION

Topically applied liposomes exhibit potential in-

- ★ eliminating local irritation
- ★ enhancing local effect
- ★ reducing or increasing systemic effect
- ★ optimizing dosage
- ★ providing prolonged release action
- ★ being cosmetically more acceptable (non-greasy and non-sticky)

The direct contact of drug with the skin is avoided when encapsulated in liposomes, so irritation potential if any, is eliminated. The concentration of free, drug depends on the release from the lipid vesicles and is always lower than in the conventional forms. The encapsulated drug is also protected from metabolic degradation. It is essential that the drug should be released from the liposomes to produce its effect, as the drug within liposomes has no access to its receptor to form "drug- receptor complex", essential for biological activity.

In order to study liposome-skin interaction, the experimental design should involve appropriate product formulation, characterization, evaluation including their biodisposition is comparison with existing commercial products or drug incorporated in a conventional dosage form.

IN VITRO SKIN PENETRATION STUDIES[17]

Cutaneous and percutaneous *in vitro* absorption studies can often produce irrelevant and useless data for actual clinical situation. The skin is most heterogeneous organ with major physiological function of a barrier. The mechanical barrier properties of skin may be maintained and simulated *in vitro* however the dynamic nature of lipid channels and the metabolic and immunologic reactions, which often serve the barrier function cannot be simulated in *in vitro* conditions. The skin controls the extent to which the intact drug penetrates into deeper layers of an through the skin. This consequently affects the actual biological activity of drug an event which cannot be assessed by *in vitro* diffusion studies. This oversight becomes even greater when*in vitro* techniques are used to investigate skin penetration of liposomal drugs. There are number of factors which influence drug diffusion *in vitro* differently than in *in vitro* conditions. In an *in vitro* experiment, the factors influencing the rate and extent of drug penetration could be controlled (e.g. temperature and moisture content) and less variables would be present (e.g. metabolic and immunologic reactions are almost non- existent). Consequently, it is easy to design and fit a pharmacokinetic model and derive mathematical equation on basis of *in vitro* experiments than on basis of *in vivo* experiments where complex multifactorial conditions exist. In the case of liposomes, factors that may lead to the destabilization of liposomal membranes and consequently release of the entrapped drug are to be considered. A high metabolic activity to breakdown the liposome phospholipids within the epidermis is expected, since the epidermal cell turnover rate is normal skin is approximately 3 to 4 weeks. When the cell reaches the stratum corneum and is keratinized, all membrane lipids are metabolised. These metabolic reactions may not be present in the dead skin tissues used in the *in vitro* experiments. In maximum *in vitro* studies receiver flask contains aqueous buffer, consequently lipid components prefer to stay in the skin tissue, which is more lipophilic. Since the presence

of liposomes or drugs within the skin samples was not analyzed, the conclusions could be valid only for transdermal rather than dermal penetration. The stability and permeability of liposomal membranes are greatly influenced by the biological environment (electrolyte, pH, proteins, biological membrane, enzymes, RES components) which is difficult if not impossible to maintain *in vitro*, especially for long periods. A change in the liposomal membrane will effect drug release from the lipid vesicles and consequently the rate of penetration of free, and encapsulated drug into and through the skin.

In vitro experiments can provide useful data only if liposomes do not penetrate the skin at all and release their content on the surface of the skin. The conditions of the skin surface can be somewhat simulated *in vitro*. Then there is no need to assess *in vivo* stability of liposomal membranes or their interaction with endogenous lipid bilayers. Human skin differs biochemically from that of the test animals, so even *in vivo* animal test results may not be relevant to clinical situations. However, animal experiments can still provide more valuable information than any *in vitro* investigations.

IN VIVO DRUG DISPOSITION STUDIES[17]

Comprehensive biodisposition studies have been conducted with a variety of liposomal products[20-22,24,39-44]. Rabbits and guinea pig were selected as test animals, the formulations (liposomal or control) were applied on a designated area in a twice-a-day multidose fashion. This is done to resemble clinical situation as well as to measure drug concentration in the various tissues when the steady state concentration was achieved (generally after 5-7 doses). Since the liposomal encapsulation alters the pharmacokinetic fate of the drug, a single dose will produce different values on the drug concentration vs time curves, consequently the sampling time could be critical. At steady-state level, the time of sampling is no longer critical. Because of skin structure diversity it is advisable to determine the drug concentration in the major layers of the skin. Separation of epidermis and dermis using enzymatic or thermal methods is not suitable here, since both allow or even enhance postmortem drug diffusion. The horizontal slicing of the skin can provide three slices:

— The first 0.2 mm slice contains mainly epidermis

— The second 0.5 mm contain only dermis

— The remaining portion mainly composed of subcutaneous tissue.

This can be easily assessed by microscopic-histological observations. Other methods which can be used to determine drug concentration at different strata include tape stripping and microtomy. The percutaneous absorption can be assessed by analyzing drug in the internal organs. The radioactive tracer technique can be used for the purpose, however, it fails to indicate the possible metabolism of active ingredients; as only isotope is measured which may be present in the drug or in its metabolite. It was observed from various biodisposition studies that liposomal formulations in multiple-dose topical treatment provided higher drug concentrations in the skin than conventional dosage forms. Certain liposome formulations enhances both dermal and transdermal drug delivery. With appropriate formulation, targeting of the drug even within the skin could be achieved.

RADIOACTIVE TRACER TECHNIQUE

It was realized that only reliable way to confirm liposomes carrier potential is to image liposomes within the skin tissue. A new electron dense marker, colloidal iron, with smaller particle size and higher concentration than colloidal gold was developed to image liposomes[45,46]. The experiments with guinea pig skin indicated presence of intact liposomes and also free colloidal iron within the dermis. The scattered iron grain probably originated from liposomes ruptured at the surface or within the skin. Such grains were also observed within the free colloidal iron

treated skin. This indicates that liposomes are absorbed on the surface of the skin, they are miscible with the surface lipids, which may act as barrier for some polar drugs. Some liposomes might rupture on the surface due to interaction with skin lipids and possibly the action of bacterial flora present there. The penetration of smaller liposomes is more likely possible via the lipid rich channels present in stratum corneum and viable dermis. It is possible that larger oligo or multilamellar liposomes lose their outer bilayers during their sojourn through skin and still carry some encapsulated drugs in the intact vesicles down to the dermis. The presence of intact liposome, within the dermis identified with help of colloidal iron strongly support this possibility. The slow clearance of drug is no only due to liposomal encapsulation but also to lack of metabolism of encapsulated drug. This obviously can lead to accumulation of drug within the skin during multiple doe therapy. The possible mechanism was also supported by electron microscopic and biodisposition[17].

EVALUATION OF LOCAL ACTIVITY

The liposome encapsulated local anaesthetics were found more effective than their conventional counter parts. This certainly indicate their carrier ability as these agents in existing commercial preparation are not effective on unbroken skin[17].

BIOCOMPATIBILITY STUDIES

A study was conducted by topical application and intramucosal injection of various liposomal products without drug using hamster as experimental animals to elucidate compatibility of liposomal components with skin[48]. The histological examination indicated that multilamellar liposomes (DPPC/chol, 2:1) were not irritant when applied topically over four days. However, 21 days of repeated topical application resulted in mild immune type reaction. Soya phosphatidyl liposomes did not show this effect, DPPC/chol liposomes are not irritant per se, but their immunogenic potential needs further investigation. Clinical investigation with soya lecithin/chol liposomes indicated no serious adverse effects. Slight erythema was observed, but it was resolved spontaneously.

MECHANISM OF TOPICAL LIPOSOMAL DRUG DELIVERY

Ultrastructural electron microscopic studies were carried out to explain the mechanism of liposomal topical drug delivery[46]. It was inferred that liposomes may penetrate the skin and serve as drug carriers, even for those drugs which otherwise would not penetrate the barrier layers of the skin. The liposomes served as a depot, as a slow release vehicle within the dermis and maintained a higher drug concentration in the skin than conventional vehicles. It was postulated that liposomal encapsulation circumvents many of the problems associated with conventional dosage forms[24] viz

- drug in the liposomal form need not be released
- diffusion of keratin layer is less of problem, as the liposomes are readily miscible with skin surface lipid
- have an excellent potential for hydrating horny layer, for the lipid vesicles create a film that supplement the skin surface lipids
- provide longer residency time for the encapsulated drug by virtue of their size
- decrease the cutaneous clearance of drug
- prevent metabolic degradation of the encapsulated drug which in turn reduce dermal/epidermal clearance of the drug
- they may also penetrate via the lipid pathways

It was also noted that diffusion of different molecules through the skin is determined by the physical properties, the molecular environment of the skin, consequently an easier diffusion of lipid soluble molecules is expected[49]. This may also imply that the diffusion of number of liposomes via this route is possible. The release of drug from the liposomes

at the skin surface and penetration of the free drug or slow release of drug from the liposomes after their penetration into the skin is confirmed by both autoradiographic and electron dense marking techniques[46]. The simulataneous occurrence of these processes is also possible. A possibility of intercellular pathways as the penetration route was supported by presence of'liposome like' material between corneocytes points. It is possible to speculate that smaller liposomes being flexible, can penetrate through the lipid channels[14,50]. It was suggested that lipophilic drug is directly transferred from the liposome to the skin, thus more drug may be delivered through the skin via liposomes[50,51]. These authors[51,52] on basis of their *in vitro* studies concluded that liposome encapsulated lipophilic drugs pass through the skin similarly to the free drug, but more polar glucose entrapped in aqueous compartment of liposomes is poorly available for transport. It was suggested that more drug can be delivered through skin via liposomes, because they allow greater pay-loads of lipophilic drugs. Such studies cannot be considered convincing as a number of factors, in addition to metabolic reactions that may lead to destabilization of liposomal membranes are to be considered which influence drug diffusion *in vitro* differently than *in vivo* conditions. The authors in their *in vitro* studies measured only percutaneous (through the skin) and not cutaneous (into the skin) penetration since skin samples were not analysed for the presence of liposomes, the conclusion could be valid only for transdermal rather than dermal penetration. Moreover, lipoid ingredients prefer to stay in the skin tissue, which is more lipophilic than the aqueous solution, mostly used in *in vitro* studies. The liposomes are very flexible, which allow them to penetrate through lipid channels with ease, which are in turn are dynamic in nature. It is also possible that phospholipids, aid to effectuate complete continuity of the epidermis, which maximally can facilitate the movement of the lipophilic drug molecules. On basis of autoradiographic and EM findings coupled with other *in vivo* experimental results, it can be postulated that[46]-

- Multi and unilamellar liposomes can be absorbed onto the skin surface intact before their penetration into the skin
- Some liposomes may rupture on the surface of skin
- The penetration of smaller vesicles is more probable
- There is possibility that intradermally localized uni or oligolamellar vesicles are derived from multilamellar liposomes that lost their outer bilayer during penetration

The ability of the stratum corneum to act as a reservoir for drug transport is amply demonstrated[53]. Several aspects related to mechanism by which liposomal entrapment enhances local activity of topically applied drugs have been revealed[30,54].

LIPOSOMES IN DERMAL DRUG DELIVERY

Corticosteroids the most frequently prescribed dermatological preparation could lead to adrenal suppression due to percutaneous absorption[55,56]. Mezei et al[21,22] reported that liposomal encapsulation of triamcinolone acetonide successfully reduced its percutaneous absorption. In another study it was concluded that liposomal formulations are improved delivery system for transdermal therapy[23]. In a series of articles, it was reported that liposomal form provided a higher drug concentration in human skin[57-60]. These studies indicate that liposomes can be used in efficient delivery of corticosteroids.

The experiments with liposomal formulation containing antifungal agents for topical application indicated that most of the liposomal products resulted in higher drug concentration in the skin and lower drug concentration in the internal organs[24,40]. In some cases both dermal and transdermal penetration was increased as a result of liposomal encapsulation. A multiphase liposomal drug delivery system was developed to selectively deliver the active ingredients within the skin tissue[40]. The system

exhibited higher patient compliance due to lack of irritation. The liposomal encapsulation of minoxidil resulted in an increased concentration within the skin layers[20,46]. It was noted that *in vivo* fate of liposome encapsulated drug is dependent on the nature of the liposomal product.

Topical local anaesthetics, which have limited penetration and short residency time, exhibited prolonged and controlled action in form of liposomal products[45]. It was observed that liposomal encapsulation of retionic acid reduced local irritation, provided greater bioavailability and higher drug concentration in the epidermis[46,61]. Topical delivery of interferon from the liposomal form was noted to be more efficient[30,62]. Preliminary clinical studies indicated that liposomes provide a suitable delivery system for interferon alpha in dermatological acceptable dosage form. In another study, it was showed that liposomes may act as sustained release vehicles for drugs into the epidermis[19]. Topical application of single dose liposome encapsulated antibiotics (Tobramycin and Silver sulfadiazine) significantly decreased bacterial counts compared to multiple dose of free drug in soft tissue infection[63].

CONCLUSION

Although there are only limited data for liposomal transdermal drug delivery, it can be speculated that liposomes can be useful for those drugs that do not penetrate the skin in the free molecular state. In such cases, liposomes can be viewed as drug carriers. They are also able to carry the drug to vascularized dermis, where it can be made available for systemic action with help of some external mean. Both biodisposition and electron microscopic investigations indicate the potential of liposomes in providing selective drug delivery that liposomes penetrate the skin carry their content into the skin, serve as slow release vehicles and reduce or increase systemic availability. A number of clinical studies have now demonstrated the superiority of liposomal drug formulations over conventional delivery systems[64]. The liposomal formulations have been successful in treatment of number of dermatological diseases and disorders such as psoriasis, mycoses, idiopathic hirsutism and cutaneous infection since they provide sustained enhanced levels in deeper strata of the skin and capable of metering a sufficient quantity of drug into deeper tissues to treat the skin symptomology[65].

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