Research Articles

Topical delivery of liposomally encapsulated gamma-interferon

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Summary

The extent of uptake of gamma interferon (γ-IFN) in various strata of hairless mouse, human and hamster skin upon application of a liposomal formulation and an aqueous solution were determined by in vitro diffusion cell experiments. For each of the animal species studied, 70–80% of the liposomally entrapped IFN was deposited onto or penetrated into the skin as determined 24 h after in vitro application. However, a significant fraction of this total amount (≈ 0.25–0.30) is either adsorbed to or associated with the stratum corneum. The drug content found in the deeper skin strata, where the receptor sites reside, suggests that drug deposition is strongly influenced by the skin species tested. The percent of applied drug found in this strata 24 h after application followed the order: hamster (6.1) $\gg$ human (0.9) $>$ hairless mouse (0.3), although the amounts of drug in the total skin of each species tested were approximately the same. This indicates that the deposition of drug into the living epidermis and/or dermis cannot be predicted by determination of the amount of drug in the total skin. The amounts in the deeper skin strata were also in the order of increasing number of follicles/hair in the skin species, suggesting that the transfollicular route is an important pathway for liposomal topical therapeutics.

Topical delivery; Gamma-interferon; Liposome

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Introduction

Gamma-interferon (γ-IFN), a major endogenously produced activation factor (MAF), is a glycoprotein secreted predominantly by sensitized T-cells in response to antigen (Murray, 1988). The recent availability of large quantities of purified γ-IFN through recombinant DNA technology (Murray et al., 1987) has generated interest in its use as a therapeutic agent for infections (Murray, 1988; Nathan et al., 1986). In the case of skin diseases, the therapeutic potential of γ-IFN may be limited by rapid clearance from the circulation and an inability to selectively deliver the molecule to the appropriate cells. When administered systemically, γ-IFN could result in undesirable side-effects and still be inadequate to suppress skin symptomology.

Liposomes have shown great potential as a drug delivery system. An assortment of molecules, including peptides and proteins, have been incorporated in liposomes, which can then be administered by different routes (Siciliano, 1985; Westerhof, 1985; Komatsu et al., 1986). Due to their high degree of biocompatibility, liposomes were initially used as delivery systems for intravenous delivery. It has since become apparent that liposomes can also be useful for delivery of drugs by other routes of administration, especially by topical application (Egbaria and Weiner, 1990).

Weiner et al. reported that topical application of liposomally entrapped alpha-interferon (α-IFN) prepared using the dehydration/rehydration method (DRV) caused a reduction of lesion scores in the cutaneous herpes simplex virus type 1 guinea pig model (Weiner et al., 1989). Egbaria et al. (1990) using in vitro studies also reported that liposomal α-IFN prepared by the DRV method was more effective in facilitating α-IFN penetration into the deeper skin strata where the basal cell layers reside. In this study, we compare the deposition of an aqueous solution and a liposomal dispersion of γ-IFN prepared using the DRV method, into various strata of mouse, hamster and human skin using in vitro diffusion cell studies.

Materials and Methods

Materials

Cholesterol (CH) and (N-[2-Hydroxyethyl]piperazine-N-[2-ethanesulfonic acid]) (HEPES) free acid were obtained from Sigma (St. Louis, MO). Egg lecithin (PC) and phosphatidylserine (PS) were obtained from Avanti Polar Lipids (Birmingham, AL). α-Tocopherol (α-T) was obtained from Eastman Kodak Co. (Rochester, NY). Lyophillized recombinant γ-IFN in vials was supplied by Genentech, Inc. (South San Francisco, CA). ^{125}I-labeled lyophilized recombinant γ-IFN was obtained from New England Nuclear corporation (Boston, MS). All solvents used were of chromatographic (high-performance liquid chromatography) grade, and the water used was double-distilled, deionized, and filtered with a Milli-Q system (Millipore Corp., Bedford, MS).

Formulations

Dehydration/rehydration liposomes (DRV) containing PC/CH/PS at a molar ratio of 1:0.5:0.1 were prepared by a modification of the method reported by Kirby and Gregoriadis (Kirby and Gregoriadis, 1984). Briefly, appropriate amounts of the various lipids, contained in a flask, were dissolved in chloroform/methanol (2:1; v/v). The total lipid concentration was 50 mg/ml and one percent α-T (an antioxidant) was added to the lipid phase of the liposomes. The solvents were removed using a rotoevaporator under vacuum at 45°C. The resultant film was dried overnight in a desiccator to remove any residual solvent. An appropriate aliquot of aqueous γ-IFN stock solution in 0.05 M HEPES buffer (pH 7.4) containing 0.1% human serum albumin and trace amounts of radiolabeled γ-IFN was then added and the mixture was hydrated at 30°C for about an hour. Intermittent vortexing was required for complete hydration. The resultant dispersion was then dehydrated at 45°C under vacuum using the rotoevaporator. When the liposomal suspension became very viscous, an amount of water, equivalent to that removed was reintroduced into the viscous suspension. The rehydrated liposomes were allowed to equilibrate for about 45 min at 30°C. The dispersion containing 100 μg/ml γ-IFN was then stored at 4°C overnight before use in the diffusion experiments.

An aqueous solution was prepared by dissolving γ-IFN in an appropriate volume of 0.05 M HEPES isotonic buffer (pH 7.4) containing 0.1% human serum albumin to obtain a solution containing 100 μg/ml γ-IFN.

Diffusion experiments

Full thickness human abdominal skin was obtained at autopsy a few hours post-mortem. Full thickness hairless mouse and hamster skin was excised from freshly sacrificed animals and immediately used after removing subcutaneous fat. The skin was mounted on a Franz diffusion cell with a nominal surface area of 2 cm^2 and a receiver compartment with a 7-ml capacity (Crown Glass, Somerville, NJ). The epidermal side of the skin was exposed to ambient conditions while the dermal side was bathed by a 0.05 M isotonic HEPES buffer (pH 7.4) containing 0.1% human serum albumin. The receiver solution was stirred continuously using a small Teflon-covered magnet. Care was exercised to remove any air bubbles between the underside of the skin and solution in the receiver compartment. The temperature of the receiver was maintained at 37°C. Following mounting of the section of skin, 200 μl of the test formulation was applied to the epidermal surface. A minimum of 3 cells were used for each formulation with each skin species. All experiments were carried out with non-occluded donor compartments. After 24 h, the experiments were stopped and the diffusion set-up was dismantled for assay of radiolabeled lipids.

Assay of radiolabeled markers

Upon dismantling, the donor compartment of the cell was rinsed carefully 5

times with 0.5 ml HEPES buffer containing 0.1% human serum albumin. The skin was then removed and it too was rinsed twice with 3 ml of the same buffer. The washing procedure was found to be sufficient to remove >99% of the formulation when determined at time zero. All washings were collected and assayed for radiolabel. Following the rinsing procedure, the skin patch was mounted on a board and a piece of adhesive tape (Scotch Magic Tape, 810, 3M Commercial Office Supply Division, St. Paul, MN), 1.9 cm wide and about 6 cm long, was used to strip the skin. The tape was of sufficient size to cover the area of skin that was in contact with the formulation. The stripping technique used was similar to that used in our earlier studies (Egbaria and Weiner, 1990). Twenty-five, ten and fifteen strippings were carried out for human, hairless mouse and hamster skin, respectively, and each strip was analyzed separately for radiolabeled drug. The donor, skin rinses, receiver solutions, the tape strippings and remaining skin were assayed for radiolabeled drug using a 5000 series Gamma Minaxi γ-counter (Packard Instrument Co. Inc., Downers Grove, IL).

A 10% solution of trichloroacetic acid in HEPES buffer (pH 7.4) was added to the samples and the contents were thoroughly mixed on a Vortex mixer (The Vortex Manufacturing Co., Cleveland, OH) and further incubated for 48 h to allow intimate contact between the protein precipitant and the γ-IFN residing in the skin tissue. The mixture was centrifuged and the supernatant (representing the amount of free iodine label) was assayed.

Results

Table 1 show the 24 h in vitro uptake of γ-IFN from the aqueous solution and the liposomal formulation in the various strata of human, hairless mouse,

TABLE 1

Distribution of γ-interferon (expressed as percent formulation applied $\pm$ S.D.; $n=3$–5) in the various strata of the skin 24 h after in vitro topical application of dehydration/rehydration liposomes to full-thickness human, hairless mouse and hamster skin[a]

Compartments	Human Skin		Hairless Mouse		Hamster Skin[b]	
	Liposomes	Aq. Sol.	Liposomes	Aq. Sol.	Liposomes	Aq. Sol.
Total Donor	22.6±0.8	90.7±3.9	20.9±0.8	91.4±1.8	29.2±0.9	77.8±1.5
Surface Stratum Corneum	57.4±3.0	5.7±2.1	32.5±5.7	6.5±3.1	30.2±3.2	11.8±0.7
Deeper Stratum Corneum	19.1±3.3	3.4±2.1	46.3±1.8	0.5±0.06	34.9±1.4	9.5±1.5
Deeper Skin Strata	0.9±0.9	0.2±0.05	0.3±0.02	0.9±0.4	6.1±2.6[c]	0.9±0.3
Total Skin	77.4±0.3	9.3±4.2	79.1±3.9	7.8±2.8	70.8±0.9	22.2±2.5
Receiver	0.01±0.0	0.03±0.0	0.08±0.1	0.8±1.4	0.00±0.0	0.02±0.0

[a] All values were corrected to 100% of recovered radioactivity.
[b] Values represent dorsal skin sections only.
[c] For abdominal skin, the value = 2.9±0.23).

hamster skin, respectively. The amount of γ-IFN adhering to the stratum corneum surface was determined by analysis of the first two tape strippings. The amount of γ-IFN penetrating the deeper stratum corneum was determined by analysis of the remaining strips (3–25 for human skin, 3–10 for hairless mouse skin, 3–15 for hamster skin). The amount of γ-IFN penetrating the deeper skin strata was determined by analysis of the remainder of the full-thickness skin.

Discussion

The efficacy of liposomal γ-IFN, prepared using the dehydration/rehydration method, compared to an aqueous γ-IFN formulation, in facilitating drug deposition was tested using hairless mouse, human and hamster skin. Liposomal α-IFN prepared by the DRV method was shown to be more effective in reducing herpes lesions in a guinea pig model (Weiner et al., 1989). The results of the 24 h in vitro studies with phospholipid DRV and aqueous γ-IFN using the three skin species shown in Table 1. It is evident from Table 1 that liposomal γ-IFN formulations are superior to aqueous formulation in facilitating drug deposition into hairless mouse, human and hamster skin. For the three skin specimens tested, the total amount of γ-IFN in the skin was about 70–80% compared to only 10–20% with an aqueous solution.

An examination of Table 1 also reveals that a significant fraction of the drug in the skin after liposomal treatment is either adsorbed to or associated with the stratum corneum. The amounts of γ-IFN deposited in the deeper skin strata after removal of the stratum corneum, appear to be strongly dependent on the nature of the skin specimen used. This is particularly evident for the drug deposition in the deeper skin strata after liposomal treatment. The amount of γ-IFN in the deeper skin strata 24 h after in vitro liposomal treatment followed the order: hairless mouse < human ≪ hamster skin. It is therefore clear that although the total amounts of γ-IFN in the three skin species were similar, the amount of drug found in the deeper skin strata (where the receptor sites reside) show dramatic differences. Thus, it is apparent from these studies that it is not possible to predict drug deposition in the deeper skin strata by determining the total amounts of drug associated with the skin. The dramatic dependence of drug deposition amounts in the deeper skin strata on the skin specimen further suggests that liposomal drug transport into strata below and beyond the stratum corneum may occur via a follicular route. To a first approximation, it is reasonable to assume that the number of follicles in a given skin specimen would be proportional to the number of hairs in that specimen. Although the absence of hair does not denote absence of follicles (such as the forehead), the presence of hair is a positive indication of the presence of follicles. Thus, hair density should be proportional to the number of hair follicles. Based on this proportionality, the follicular pathway would be in the order: hamster ≫ human > hairless mouse. Further, it is well known that the follicles in mice,

rendered hairless genetically, are not fully developed and may be in a state of atrophy. It is also clear that the number of follicles in human abdominal skin is quite low compared to that in the forehead, back, postauricular area or the forearms (Szabo, 1958). An examination of the amounts of γ-IFN deposited from liposomal formulations in the deeper skin strata of hamster skin reveals some interesting trends. It is found that the amounts are higher when dorsal skin is used compared to abdominal skin. These differences may be related to the number and density of follicles in the skin specimen. It is also clear that even for abdominal skin, wherein the follicular density is lower, liposomal formulations allow substantially greater deposition of γ-IFN in the deeper skin strata compared to that from aqueous solutions. Considering all the above factors, we believe that the number of follicles in the skin species tested is in the order: hamster $\gg$ human $>$ hairless mouse skin. Differences in percutaneous absorption of a variety of drugs in various animal species have been discussed in terms of differences in follicular densities (Barry, 1983).

We have also recently demonstrated that the follicular route is an important pathway in the transport of drugs from liposomal formulations (Weiner et al., 1991). Although the relative importance of the lipoidal or stratum corneum-mediated pathway and the follicular pathway in facilitation-enhanced permeation of drugs such as γ-IFN is still unclear, it is probable that the latter pathway might be predominantly responsible, especially because a molecule such as γ-IFN is not expected to readily permeate the stratum corneum due to its size (molecular weight) and hydrophilicity. The proportionality of γ-IFN amounts in the deeper skin strata of various skin species with the estimated follicular density therefore appears to corroborate such a pathway for γ-IFN transport from liposomal formulations.

It is recognized that differences in stratum corneum lipid compositions and thicknesses may play some role in determining such differences. However, the major differences in drug deposition in the deeper skin strata appears to be closely related to the size and density of follicles in the skin sample.

The importance of the lipoidal pathway should not be minimized, however. When an applied liposomal drug suspension begins to dry, dehydration transforms the liposomes into lipid bilayer structures that adhere or bind strongly to the surface of the skin. Such binding may occur between liposomal lipid bilayers and corneocytes or between liposomal and stratum corneum bilayers. The adhesive strength appears to be quite high judging from the inability of simple rinsing procedures to dislodge a significant portion of the applied liposomal lipids. The 'surface' liposomal bilayers containing drug provide a drug reservoir allowing sustained release of the drug, specifically small lipophilic drugs such as hydrocortisone, across the stratum corneum into the dermis and blood vasculature (Jacobs et al., 1988; Gesztes and Mezei, 1988). The liposomal lipid bilayers formed on the surface after dehydration present an additional and substantial barrier of drug diffusion. It is possible, therefore, to control drug release into and across skin by controlling the amounts of liposomal lipids applied.

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