Topical Delivery of Liposomally Encapsulated Interferon Evaluated in a Cutaneous Herpes Guinea Pig Model

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The topical delivery of liposomally encapsulated interferon was evaluated in the cutaneous herpes simplex virus guinea pig model. Application of liposomally entrapped interferon caused a reduction of lesion scores, whereas application of interferon formulated as a solution or as an emulsion was ineffective. The method of liposomal preparation rather than the lipid composition of the bilayers appeared to be the most important factor for reducing lesion scores. Only liposomes prepared by the dehydration-rehydration method were effective. This finding implied that the dehydration and subsequent rehydration of the liposomes facilitate partitioning of the interferon into liposomal bilayers, where the drug is positioned for transfer into the lipid compartment of the stratum corneum. Liposomes do not appear to function as permeation enhancers but seem to provide the needed physicochemical environment for transfer of interferon into the skin.

Recurrent herpes simplex labialis has been reported to affect almost one-half of the population of the United States, and 25% of those affected have frequent or severe recurrences of both (39). Genital herpes, though not officially recognized as a disease until 1966, is currently an epidemic sexually transmitted disease. Although many forms of therapy have been tested (29, 37), none has proven profoundly beneficial in decreasing the severity and frequency of the clinical manifestations of the disease. After infection, herpesvirus particles retreat into the regional ganglia and establish a latent state. They are inaccessible to therapy in their ganglionic residence; therefore, the control of herpes labialis rests on our ability to suppress symptoms promptly as the virus begins reactivation. It appears that this could best be done by delivering an effective antiviral agent into the living epidermal tissues of the skin during the prodromal stage of lesion development.

It is believed that virus replication and lateral spread in the basal layer can be arrested during the prodromal stage (33, 37) by either chemotherapeutic or immunotherapeutic drugs. Of the latter class of agents, interferon (IFN) seems to have especially high potential for the treatment of herpes, condylomata acuminata, and other similarly manifested viral disease states (9, 32). Data from a number of recent clinical studies demonstrate that IFN is most effective when used prophylactically rather than clinically for both genital warts and herpes infections (18, 25, 32).

When systemic routes of administration are used to deliver drugs to extravascular sites, sufficient drug must be administered to account for the distribution of the drug through all tissues of the body. Therefore, systemic regimens adequate to suppress skin symptomatology often result in adverse systemic effects and still may not overcome the inaccessibility of the target tissue to the drug. In these regards, drug delivery remains the most limiting factor to the effective treatment of herpes.

Recently, much attention has been focused on liposomes in the search for a more effective means of delivering intrinsically active drugs to their tissue targets, and lipo-

somes are being viewed as potential carriers for site-directed delivery of drugs such as insulin, enzymes, antimicrobial agents, antitumor agents, and biological-response modifiers (13, 24, 30, 31, 34). Recent reports from a number of laboratories have dealt with the physicochemical properties of IFN-containing liposomes and the resultant antiviral activities of this drug. Both fibroblast and leukocyte IFNs have been incorporated successfully into liposomes (7, 8). These studies show that the physical location and the extent of incorporation of IFN into liposomes as well as the stability of IFN and antiviral activity are dependent on lipid composition of the liposome.

The successful treatment of cutaneous virus infections with IFN relies on an ability to effectively deliver IFN to the infected cells. So far, it has proven impossible to obtain adequate levels in tissue to control herpes and other skin viruses by conventional avenues of drug administration. Mezei and Gulasekharan (27, 28) suggested that liposomes be used as drug carriers by the topical route. Subsequently, we have shown that while liposomes themselves do not penetrate intact skin, they facilitate deposition of drugs which tend to associate with liposomal bilayers into the skin (15, 19). The present study evaluates the topical delivery of liposomally encapsulated IFN in the cutaneous herpes simplex virus (HSV) guinea pig model.

MATERIALS AND METHODS

Egg lecithin (EL), cholesterol (CH), cholesteryl sulfate (CHS), bovine brain ceramides (CM), palmitic acid (PA), and dimyristoylphosphatidylcholine (DMPC) were obtained from Sigma Chemical Co. (St. Louis, Mo.). Phosphatidylserine (PS) was obtained from Avanti Polar Lipids (Birmingham, Ala.). α -Tocopherol was obtained from Eastman Kodak Co. (Rochester, N.Y.). Lyophilized recombinant leukocyte A IFN in vials, each containing 18×10^6 IU of IFN, 9 mg of sodium chloride, and 5 mg of human serum albumin, was supplied by Hoffmann-La Roche Inc. (Nutley, N.J.). CH was recrystallized twice from ethanol. All other compounds were used as received. The S-148 strain of HSV type 1 (HSV-1) was provided by T. W. Schafer of Schering Corp., Bloomfield, N.J. Titration was performed by plaque reduction in BSC-1 cells as described previously (38).

IFN formulations. Three types of liposomes were tested.

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In all cases, the final volume was adjusted so that the concentration of total lipid was 100 $\mu\text{mol/ml}$. The ratio of IFN to human serum albumin was maintained at 4×10^6 IU/mg, and the final alpha IFN (IFN- α) concentration was 5.4×10^6 IU/ml of suspension. The effect of lipid composition was tested by preparing liposomes of EL-CH-PS and DMPC-CH-PS at molar ratios of 2:1:0.33. α -Tocopherol (an antioxidant [1%]) was added to all liposomes containing EL. Liposomes were also prepared from lipids with compositions similar to those found in the stratum corneum (CM-CH-PA-CHS; 4:2.5:2.5:1, molar ratio). Positively charged liposomes were not tested, since EL-CH-stearylamine (2:1:0.33) liposomes were more irritating than the neutral or negatively charged liposomes (they caused a slight redness with twice daily application for 3 days). Neutral liposomes were not tested, since they flocculated within 1 week of storage. With a phase-contrast microscope, the flocculation could be observed as large aggregates. An aqueous IFN solution and an IFN-containing water-in-oil emulsion (mineral oil-buffered solution-Arlacel 80, 6:3:1 [wt/wt]) were also tested.

MLV. Multilamellar liposomes (MLV) were prepared by standard procedures. Briefly, the various lipid mixtures tested were dissolved in chloroform and rotary evaporated under nitrogen. The flask containing the thin lipid film was then stored overnight under vacuum to facilitate removal of residual solvent. The lipid film was then resuspended (at a temperature above the phase transition temperature of the phospholipid) in calcium- and magnesium-free phosphate-buffered saline (pH 7.0) containing IFN and human serum albumin, and the mixture was vortexed for 10 to 30 min. No attempt was made to remove free drug, since leakage from the aqueous liposomal compartment is minimized when the external thermodynamic activity of the drug approximates its thermodynamic activity in the aqueous compartments of the liposome.

LUV. Large unilamellar vesicles (LUV) were prepared with an extrusion apparatus (The Extruder; Lipex Biomembranes Inc., Vancouver, British Columbia, Canada) fitted with 100-nm-pore-size polycarbonate membrane filters (Nuclepore Corp., Pleasanton, Calif.). The method is based on observations that as MLV are repeatedly extruded through 0.1- μm -pore-diameter polycarbonate membranes under 250 lb of pressure per in^2 , their average diameter becomes progressively smaller, reaching a minimum of 100 nm after about 5 to 10 extrusions (20). As the MLV are forced through the pores, successive layers are peeled, until one intact bilayer remains.

DRV. DRV were prepared by the method of Kirby and Gregoriadis (23). Briefly, empty sonicated vesicles were mixed with an aliquot of IFN stock solution, and the mixture was dried under a stream of nitrogen. During dehydration, the small vesicles fused to form a multilamellar film that effectively sandwiched the solute molecules between successive bilayers. Upon rehydration, large vesicles which had encapsulated a significant proportion of the solute were produced. This method has potential application to large-scale production, since it would depend exclusively on controlled drying and rehydration processes and would not require extensive use of organic solvents (the IFN is never in contact with organic solvent), detergents, or dialysis systems.

Experimental animals and cutaneous HSV-1 infection model. The test for reduction of HSV-1 lesions was a modification of the procedure described by Shipman et al. (35). Results from initial experiments using conventional guinea pigs were erratic because of changes caused by

shaving and depilation procedures. Therefore, all of the cutaneous guinea pig model experiments were performed with hairless guinea pigs [CrI:IAF(HA)BR] supplied by Charles River Breeding Laboratories, Inc., Wilmington, Mass. This seemed reasonable, since the permeability barrier of hairless guinea pig skin is similar to that of human skin when tested with hydrocortisone as a marker in Franz diffusion cells (unpublished data). Seven adult female hairless guinea pigs, weighing 300 to 400 g each, were anesthetized with Innovar-Vet (Pitman-Moore), and the backs were divided into six squares with a marking pen. S-148 HSV-1 (25 μl) at a titer of 3×10^7 PFU/ml was applied in the center of each square. The virus was inoculated with a spring-loaded vaccination instrument (Sterneedle Gun; Panray Division, Ormont Drug Co., Englewood, N.J.) which was released 10 times, producing inoculations 0.75 mm deep on each skin area. Generally, the three areas on the left or right sides of each animal were treated topically three times daily with 50 μl of formulation for 5 days beginning at ~ 20 h after inoculation. The contralateral sites generally received either no treatment or vehicle alone and served as control sites. To quantitate the effects of formulation factors on therapeutic efficacy (lesion score), the scoring system of Alenius and Oberg (3) was used. After inoculation of the guinea pigs with HSV as described above, the inoculated areas were scored daily for 9 to 10 days. All scoring was done blind. Time to healing was also noted for each drug. A statistical procedure based on programs available in the Michigan Interactive Data Analysis System (12) was developed to analyze the data, using techniques of profile analysis, paired *t* tests, and analysis of variance. This procedure, as described by Shipman et al. (35), was used to determine if each of the formulations tested produced a statistically significant reduction in total lesion score.

RESULTS

No significant drug activity was apparent upon topical application of either the IFN solution (Fig. 1a) or the IFN emulsion (Fig. 1b). IFN entrapped in EL-CH-PS (2:1:0.33) MLV (Fig. 1c) and LUV (Fig. 1d) was also ineffective. On the other hand, IFN entrapped in EL-CH-PS (2:1:0.33) and DMPC-CH-PS (2:1:0.33) liposomes (Fig. 1e and f, respectively) prepared by the dehydration-rehydration method was superior to controls with respect to reduction of lesion scores. Finally, when IFN was entrapped in DRV liposomes prepared from lipids of a composition similar to that of the stratum corneum (CM-CH-PA-CHS, 4:2.5:2.5:1), efficacy was observed (Fig. 1g). When a formulation containing empty liposomes of the same composition in combination with free IFN was tested, no efficacy was seen (Fig. 1h).

DISCUSSION

Over the last 5 years, mixed results have been obtained when herpes infections and genital warts have been treated with topical formulations of IFN. Grebeniuk et al. (17) reported that an ointment containing pig leukocytic IFN had a pronounced therapeutic effect in herpetic infections of the face skin and genitalia. Similarly, Ikic et al. (21) reported that treatment of labial and genital herpes with human leukocyte IFN contained in ointments resulted in less frequent relapses and smaller lesions. In neither of these studies is the composition of the ointment stated, making it difficult to evaluate the significance of the clinical data. Friedman-Kien et al. (14) reported improvement of clinical

FIG. 1. Effect of various IFN-containing formulations on HSV-1 lesions in the cutaneous herpes guinea pig model. Treatment commenced ~20 h after virus inoculation and consisted of three daily applications of 50- μ l formulation for 5 days. Data are averages of results taken from seven hairless guinea pigs $\pm$ standard error of means. For details, see Materials and Methods. The following formulations, each containing 5.4×10^6 IU of IFN per ml were tested: (a) aqueous solution, (b) water-in-oil emulsion, (c) EL-CH-PS MLV, (d) EL-CH-PS LUV, (e) EL-CH-PS DRV; (f) DMPC-CH-PS DRV, (g) CM-CH-PA-CHS DRV containing entrapped IFN, and (h) CM-CH-PA-CHS DRV containing free IFN.

indications such as ending of new-lesion formation, scabbing, and healing of lesions in patients with recurrent genital herpes who were treated with a combination of IFN- α and the surfactant nonoxynol-9 in a cream base. Finally, Glezerman et al. (16) reported that an IFN- β carboxymethyl cellulose gel base formulation applied directly to herpes eruptions for 10 days reduced the mean number of recurrences and the duration of eruptions in patients with either genital herpes or herpes labialis.

In contrast, clinically disappointing results of topical IFN therapy have been reported. Eron et al. (10), in a study of 94 patients with recurrent genital herpes, reported that application of aqueous solutions of IFN- α -2a to unroofed vesicles resulted in no difference between the placebo and the drug-containing formulation with respect to duration of viral shedding, time to crusting, and time to healing of herpetic lesions. The authors pointed out that since the clinical efficacy of topically administered acyclovir (22, 36) is dependent on the ability of acyclovir to penetrate the skin, which is in turn dependent on the vehicle used, the lack of efficacy of the IFN formulation may well have been the result of the inability of the vehicle (a simple aqueous solution) to facilitate transport of IFN through the skin. A recent study by Freeman et al. (13) supports the premise that efficacy or lack thereof is coupled to the delivery system and the timing of its use. When the guinea pig model was used, marginal efficacy was observed with different IFN-containing formulations (solutions, emulsions, creams, and gels) when treatments were initiated 24 h prior to virus inoculation. In contrast, there was no efficacy when these formulations were applied subsequent to virus inoculation. Therefore, failure of the solution (Fig. 1a) and emulsion (Fig. 1b) formulations used in this study to demonstrate significant reduction of lesion scores is not unexpected.

We (15) have recently demonstrated that liposomally encapsulated lipophilic drugs like progesterone and hydrocortisone, which are intercalated within the bilayer structure of the lipids in MLV, pass through the skin with a facility comparable to that of free drug. On the other hand, highly polar glucose entrapped in the aqueous compartments of the liposome is not readily available for transport. Theoretical analysis of the data (19) supports the concept that direct transfer of drug from liposome to skin occurs only when the drug is intercalated within the bilayer. The failure of the MLV (Fig. 1c) and LUV (Fig. 1d) formulations to elicit a pharmacological response suggests that the entrapped IFN does not penetrate the lipid bilayers and is thus unavailable for transfer into the lipid domains of the skin. This speculation is consistent with monolayer experiments (N. A. Williams and N. D. Weiner, *Int. J. Pharm.*, in press) which indicate that, while IFN does not penetrate into deep compartments of the liposomal bilayers, it does accumulate at and associate with the surface of bilayers.

Perhaps the most intriguing outcome of this study is the observation that liposomes, when prepared by the dehydration-rehydration method (Fig. 1e through g), significantly reduced lesion scores. These data, particularly the comparison of Fig. 1 through e, indicate that the method of liposomal preparation, rather than the lipid composition of the bilayers, is the most important factor in reducing lesions in the cutaneous guinea pig model. This finding implies that the dehydration and subsequent rehydration of the liposomes facilitates partitioning of the IFN into the liposomal bilayer at a point where it is positioned for transfer into the lipid compartment of the stratum corneum. Preliminary in vitro diffusion experiments, using intact hairless guinea pig

skin as the membrane, indicate that IFN in an aqueous formulation does not permeate intact skin, even when permeation enhancers (Azone and dimethyl sulfoxide) are used. Consistent with the in vivo antiviral activity of IFN-containing DRV, we found that IFN was transported through intact guinea pig skin (~1% in 48 h) only when it was incorporated in liposomes prepared by the dehydration-rehydration method (unpublished data). We are presently pursuing this aspect of the study. Although DRV entrap approximately 20% IFN, whereas MLV and LUV entrap only about 10% IFN (as determined by the Campbell et al. procedure [6]), we have thus far been unable to determine the partition coefficient of IFN between the bilayers and the aqueous compartments in the various liposomal systems. Since we are uncertain as to the location of the IFN entrapped in each type of liposome, the relationship between antiviral activity and degree of entrapment for the three liposomal preparations remains unresolved.

Unlike virtually all other biologic membranes, the stratum corneum does not contain phospholipids. It is made primarily of CM (40%), CH (25%), fatty acids (primarily PA; 25%) and CHS (10%). Lipid compositions similar to those given above have been used to form stable liposomes (1, 2, 26, 40), and preliminary work in our laboratories confirms these observations. While such studies have been useful in elucidating physicochemical characteristics of the stratum corneum, especially concerning its bilayer properties, there are no published reports about such liposomes being tested as drug delivery systems. If transfer of drug from liposomal bilayers to stratum corneum bilayers is an essential aspect of the mechanism by which liposomal entrapment facilitates drug transport into the skin, it seems logical that the use of "skin lipid" liposomes would expedite this transfer. The fact that the CM-CH-PA-CHS formulation (Fig. 1g) appears to be even more efficacious than the phospholipid-based DRV thus far tested tends to support this assumption. These skin lipid liposome data are particularly encouraging, since no attempt was made to optimize the lipid ratios.

Figure 1h shows that the skin lipid liposomes are ineffective when only free rather than entrapped IFN is present. This is a particularly important control experiment, since it is possible that the mere presence of these lipids in the skin could alter the barrier properties of the stratum corneum, allowing enhanced transport of free drug. It has been reported that modification of the lipid composition of the skin by treatment with permeation enhancers, including PA, results in substantial enhancement of drug permeation (4). Although the exact mechanism by which permeation is enhanced remains uncertain, data suggest that these agents may alter bilayer packing arrangements, resulting in a more fluid and permeable membrane (4, 5). A comparison of Fig. 1g and h strongly suggests that liposomes do not function to enhance permeation in this sense. Rather, they appear to provide the needed physicochemical environment for IFN to transfer into the skin. The effect appears to be a unique cooperative drug-enhancer effect.

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LITERATURE CITED

1. Abraham, W., P. W. Wertz, and D. T. Downing. 1988. Fusion patterns of liposomes formed from stratum corneum lipids. *J. Invest. Dermatol.* 90:259-262.

2. Abraham, W., P. W. Wertz, L. Landmann, and D. T. Downing. 1987. Stratum corneum lipid liposomes: calcium-induced transformation into lamellar sheets. *J. Invest. Dermatol.* **88**:212-214.
3. Alenius, S., and B. Oberg. 1978. Comparison of the therapeutic effects of five antiviral agents on cutaneous herpesvirus infection in Guinea pigs. *Arch. Virol.* **58**:277-288.
4. Aungst, B. J., N. J. Rogers, and E. Shefter. 1986. Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. *Int. J. Pharm.* **33**:225-234.
5. Beastall, J. C., J. Hadgraft, and C. Washington. 1988. Mechanism of action of Azone as a percutaneous penetration enhancer: lipid bilayer fluidity and transition temperature effects. *Int. J. Pharm.* **43**:207-213.
6. Campbell, J. D., T. Grunberger, M. A. Kochman, and S. L. White. 1975. A microplaque reduction assay for human and mouse interferon. *Can. J. Microbiol.* **21**:1247-1255.
7. Eppstein, D. A. 1982. Altered pharmacological properties of liposome-associated human interferon-alpha II. *J. Interferon Res.* **2**:117-125.
8. Eppstein D. A., and W. E. Stewart II. 1981. Binding and capture of human interferon-alpha by reverse evaporation vesicles, multilamellar vesicles, and small unilamellar vesicles. *J. Interferon Res.* **1**:495-504.
9. Eron, L. J., F. Judson, S. Tucker, S. Praver, J. Mills, K. Murphy, and M. Hickey. 1986. Interferon therapy for condylomata acuminata. *N. Engl. J. Med.* **315**:1059-1064.
10. Eron, L. J., C. Toy, B. Salsitz, R. R. Scheer, D. L. Wood, and P. I. Nadler. 1987. Therapy of genital herpes with topically applied interferon. *Antimicrob. Agents Chemother.* **31**:1137-1139.
11. Fountain, M. W., C. Dees, and P. Schultz. 1981. Enhanced intracellular killing of *Staphylococcus aureus* by canine monocytes treated with liposomes containing amikacin, gentamicin, kanamycin, and tobramycin. *Curr. Microbiol.* **6**:373-376.
12. Fox, D. J., and K. E. Guire. 1973. MIDAS: Michigan Interactive Data Analysis System: 59-60. The Statistical Research Laboratory of The University of Michigan, Ann Arbor.
13. Freeman, D. J., M. B. McKeough, and S. L. Spruance. 1987. Recombinant human interferon- α A treatment of an experimental cutaneous herpes simplex virus type 1 infection of guinea pigs. *J. Interferon Res.* **7**:213-222.
14. Friedman-Kien, A. E., R. J. Klein, R. D. Glaser, and S. M. Czelusniak. 1986. Treatment of recurrent genital herpes with topical alpha interferon combined with nonoxyl 9. *J. Am. Acad. Dermatol.* **5**:989-994.
15. Ganesan, M. G., N. D. Weiner, G. L. Flynn, and N. F. H. Ho. 1984. Influence of liposomal drug entrapment on percutaneous absorption. *Int. J. Pharm.* **20**:139-154.
16. Glezerman, M., E. Lunenfeld, V. Cohen, I. Sarov, M. Movshovitz, T. Doerner, J. Shoham, and M. Revel. 1988. Placebo-controlled trial of topical interferon in labial and genital herpes. *Lancet* **ii**:150-152.
17. Grebeniuk, V. N., V. I. Marchenko, V. V. Parfenov, and N. A. Zubanova. 1983. Clinical study of the therapeutic effectiveness of an ointment consisting of swine leukocytic interferon. *Antibiotiki* **28**:848-852.
18. Gross, G., H. Ikenberg, A. Roussaki, N. Drees, and E. Schopf. 1986. Systematic treatment of condylomata acuminata with recombinant interferon-alpha-2a: low dose superior to high dose regimen. *Chemotherapy (Basel)* **32**:537-541.
19. Ho, N. F. H., M. G. Ganesan, N. D. Weiner, and G. L. Flynn. 1985. Mechanisms of topical delivery of liposomally entrapped drugs. *J. Cont. Release* **2**:61-65.
20. Hope, M. J., M. B. Bally, G. Webb, and P. R. Cullis. 1985. Production of large unilamellar vesicles by a rapid extrusion procedure. *Biochim. Biophys. Acta* **812**:55-65.
21. Ikic, D., D. Trajer, K. Cupak, I. Petricevic, M. Prazic, I. Soldo, D. Jusic, S. Smerdel, and E. Soos. 1981. The clinical use of human leukocyte interferon in viral infections. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **19**:498-505.
22. Kinghorn, G. R., E. B. Turner, I. G. Barton, C. W. Polter, C. A. Burke, and A. P. Fiddian. 1983. Efficacy of topical acyclovir cream in first and recurrent episodes of genital herpes. *Antiviral Res.* **3**:291-301.
23. Kirby, C. J., and G. Gregoriadis. 1984. A simple procedure for preparing liposomes capable of high encapsulation efficiency under mild conditions, p. 19-28. In G. Gregoriadis (ed.), *Liposome technology*, vol. 1. CRC Press, Inc., Boca Raton, Fla.
24. Koff, W. C., S. D. Showalter, B. Hampar, and I. J. Fidler. 1985. Protection of mice against fatal herpes simplex type 2 infection by liposomes containing muramyl tripeptide. *Science* **228**:495-497.
25. Kuhls, T. L., J. Sacher, E. Pineda, D. Santomauro, E. Wiesmeier, W. A. Growdon, and Y. J. Bryson. 1986. Suppression of recurrent genital herpes simplex virus infection with recombinant alpha 2 interferon. *J. Infect. Dis.* **154**:437-442.
26. Landmann, L. 1984. The epidermal permeability barrier. Comparison between in vivo and in vitro structures. *Eur. J. Cell Biol.* **33**:258-264.
27. Mezei, M., and V. Gulasekharan. 1980. Liposomes—a selective drug delivery system for the topical route of administration: lotion dosage form. *Life Sci.* **26**:1473-1477.
28. Mezei, M., and V. Gulasekharan. 1982. Liposomes—a selective drug delivery system for the topical route of administration: gel dosage form. *J. Pharm. Pharmacol.* **34**:473-474.
29. National Institutes of Health. 1986. Sexually transmitted diseases: 1986 NIAID study group summary and recommendations. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Washington, D.C.
30. Parker, R. J., E. R. Priester, and S. M. Sieber. 1982. Comparison of lymphatic uptake, metabolism, excretion, and biodistribution of free and liposome-entrapped cytosine arabinofuranoside following intraperitoneal administration to rats. *Drug Metab. Dispos.* **10**:40-46.
31. Patel, K. R., M. H. Jonah, and Y. E. Rahman. 1982. In vitro uptake and therapeutic application of liposome-encapsulated methotrexate in mouse hepatoma 129. *Eur. J. Cancer Clin. Oncol.* **18**:833-839.
32. Pazin, G. G., J. H. Harger, J. A. Armstrong, M. K. Breinig, R. J. Caplan, K. Cantell, and M. Ho. 1987. Leukocyte interferon for treating first episodes of genital herpes in women. *J. Infect. Dis.* **156**:891-898.
33. Pazin, G. J., J. A. Armstrong, M. T. Lam, G. C. Tarr, P. J. Jannetta, and M. Ho. 1979. Prevention of reactivated herpes simplex infection by human leukocyte interferon after operation on the trigeminal root. *N. Engl. J. Med.* **301**:225-230.
34. Ryman, E. B., R. F. Jewkes, K. Jeyasingh, M. P. Osborne, H. M. Patel, V. J. Richardson, M. H. N. Tattersall, and D. A. Tyrrell. 1978. Potential application of liposomes to therapy. *Ann. N.Y. Acad. Sci.* **308**:281-307.
35. Shipman, C., Jr., S. H. Smith, J. C. Drach, and D. L. Klayman. 1986. Thiosemicarbazones of 2-acetylpyridine, 2-acetylquinoline, 1-acetylisquinoline, and related compounds as inhibitors of herpes simplex virus *in vitro* and in a cutaneous herpes guinea pig model. *Antiviral Res.* **6**:197-222.
36. Spruance, S. L., M. B. McKeough, and J. R. Cardinal. 1984. Penetration of guinea pig skin by acyclovir in different vehicles and correlation with the efficacy of topical therapy of experimental cutaneous herpes simplex virus infection. *Antimicrob. Agents Chemother.* **25**:10-15.
37. Tummon, I. S., D. F. Dudley, and J. H. Walters. 1981. Genital herpes simplex. *Can. Med. Assoc. J.* **125**:23-39.
38. Turk, S. R., C. Shipman, Jr., R. Nassiri, G. Genzlinger, S. H. Krawczyk, L. B. Townsend, and J. C. Drach. 1987. Pyrrolo[2,3-*d*]pyrimidine nucleosides as inhibitors of human cytomegalovirus. *Antimicrob. Agents Chemother.* **31**:544-550.
39. U.S. Department of Health and Human Services. 1980. Summary and recommendations of the NIAID study group and sexually transmitted diseases. U.S. Government Printing Office no. 0-311-201/3052. U.S. Department of Health and Human Services, Washington, D.C.
40. Wertz, P. W., W. Abraham, L. Landmann, and D. T. Downing. 1986. Preparation of liposomes from stratum corneum lipids. *J. Invest. Dermatol.* **87**:582-584.