Isfahan University of Medical Sciences

School of Pharmacy and Pharmaceutical Sciences

Department of Pharmaceutics

Isfahan Pharmaceutical Sciences Research Center

Ph.D Thesis in Pharmaceutics

Research project No. 79340

Title:

Liposome-mediated Targeting of Finasteride to the Hair Follicles

Supervised by:

Majid Tabbakhian (Ph.D)

Naser Tavakoli (Ph.D)

Mahmoud Reza Jaafari (Ph.D)

by:

Saeid Daneshamouz (Pharm.D)

July 2005

Liposome-mediated targeting of finasteride to the hair follicles

Abstract

Introduction: Targeted delivery of finasteride, indicated orally in the treatment of alopecia and some other pilosebaceous disorders, to the hair follicles can increase the concentration of the drug at pilosebaceous units (PU) while reducing its systemic side effects.

Methods: Finasteride-entrapped multilamellar liposomes/niosomes (MLVs) were prepared by the film formation and reverse evaporation methods. The MLVs were characterized with regard to the size, drug entrapment efficiency, and release behavior in various media and drugs permeability through different membranes. *In vivo* deposition of ^{3}H-finasteride into PU and other compartments of the hamster ear was determined following topical application of finasteride-MLVs and a hydroalcoholic finasteride solution (HA) by scraping method. The decrease in the area of the ear and flank sebaceous glands after twice a day/4 weeks topical application was quantitatively determined by planimetry of histological sections of skin.

Results: Finasteride permeation through hamster flank skin was faster from finasteride-HA than from finasteride-vesicles (0.13 vs. 0.025-0.058 $\mu g/cm^2.h$, respectively), ($p<0.05$). The $T_{50\%}$ release from liquid-state liposomes/niosomes into artificial sebum at 37 °C ranged from 2.5 to 7.5 h. Un-extruded charged MLVs comprising liquid-state amphiphiles of Brij97, dimyristoyl phosphatidylcholine (DMPC), Brij97:Brij76 (1:1 mole ratio) deposited 2.05-2.5% of the applied dose to the

PU. This was significantly higher than 0.35-0.92%, which was deposited by extruded vesicles (100, 400 nm) of the same liquid-state amphiphiles, gel-state vesicles, or finasteride-HA. Selected finasteride-MLVs, HA of finasteride and a hydroalcoholic progesterone solution (positive control) significantly decreased the size of PU of ear and flank organ located under application site (36-48%, $p<0.01$), while HA of finasteride exhibited a systemic effect as determined by measuring the PU size in untreated side of the flank ($p<0.05$).

Conclusion: Both *in vivo* deposition and *in vivo* bioassay studies demonstrated the potential of charged liquid-state MLVs for effective delivery of finasteride to the PU.

Key words: Finasteride, niosome, liposome, deposition and targeting, pilosebaceous unit, hamster ear and flank

Table of content

CHAPTER 2: MATERIALS, INSTRUMENTS AND METHODS

List of Tables

List of Figures

List of Appendices

List of abbreviations

Chol	Cholesterol
CPM	Count Per Minute
CPP	Critical Packing Parameter
DCP	Dicethyl phosphate
DHT	Dihydro testosterone
DMPC	Dimyristoyl phosphatidylcholine
DPM	Disintegration Per Minute
DPPC	DIPALMITOYLPHOSPHATIDYLECHOLINE
DSC	Differential Scanning Calorimetry
EE	Entrapment Efficiency
EO	Ethylene Oxide
Fin.	Finasteride
GDL	Glyceryl di-laurate
GDS	Glyceryl di-stearate
HA	Hydroalcoholic solution
HSDSC	High sensitivity differential scanning calorimetry
LOD	Limit Of Detection
LOQ	Limit Of Quantitation
LUV	Large Unilamellar Vesicles
m. r.	Molar ratio
MLV	Multi Lamellar Vesicles
Mol%	Mole percent
NSVs	Non-ionic surfactant vesicles
PBS	Phosphate Buffered Saline
PC	Egg Phosphatidylcholine
PG	Propylene Glycol
POE	Polyoxyethylene
Prog.	Progesterone
PU	Pilosebaceous unit
RT	Room Temperature
SC	Stratum Corneum
SA	stearylamine
T	Testosterone
T_c	Transition Temperature
TEM	Transmission Electron Microscopy

References

1. Lauer AC, Lieb LM, Ramachandran C, Flynn GL, Weiner N. Transfollicular drug delivery. Pharm Res 1995;12(2):179-86.

2. Gupchup GV, Zata J. Targeted delivery to pilosebaceous structures. Cosmetics and Toiletries 1997;112:79-88.

3. Osborne DW, Hatzenbuhler DA. The Influence of skin surface lipid on topical formulations. In: Osborne DW, Amann AH, editors. Topical Drug Delivery Formulations.New York: Marcel Dekker Inc., 1990.

4. Grams YY, Bouwstra JA. Penetration and distribution of three lipophilic probes in vitro in human skin focusing on the hair follicle. J Controlled Release 2002;83:253-62.

5. Lauer AC, Ramachandran C, Lieb LM, Niemiec SM, Weiner N. Targeted delivery to the pilosebaceous unit via liposomes. Adv Drug Deliv Rev 1996;18:311-24.

6. Niemiec SM, Ramachandran C, Weiner N. Influence of nonionic liposomal composition on topical delivery of peptide drug into pilosebaceous units: an in vivo study using the hamster ear model. Pharm Res 1995;12(8):1184-8.

7. Weiner N. Targeted follicular delivery of macromolecular via liposomes. Int J Pharm 1998;162:29-38.

8. Rolland A, Wagner N, Chatelus A, Shroot B, Schaefer H. Site-specific drug delivery to pilosebaceous structures using polymeric microspheres. Pharm Res 1993;10(12):1738-44.

9. Sumian CC, Pitre FB, Gauthier BE, Bouclier M, Mordon SR. A new method to improve penetration depth of dyes into the follicular duct: potential application for laser hair removal. J Am Acad Dermatol 1999;41:172-5.

10. Tata S, Weiner N, Flynn G. Relative influence of ethanol and propylene glycol cosolvents on deposition of minoxidil into the skin. J Pharm Sci 1994;83:1508-10.

11. Touitou E, Godin B, Weiss C. Enhanced delivery of drugs into and across the skin by ethosomal carriers. Drug Dev Res 2000;50:406-15.

12. Wu HL, Ramachandran C, Weiner N, Roessler BJ. Topical transport of hydrophilic compounds using water-in-oil nanoemulsions. Int J Pharm 2001;220:63-75.

13. Lieb LM, Liimatta AP, Bryan RN, Krueger GG. Description and definition of a transfollicular route of permeant for topically applied agents to human scalp skin. J Invest Dermatol 1995;104:655.

14. Hueber F, Wepierre J, Schaefer H. Role of transepidermal and transfollicular routes in percutaneous absorption of hydrocortisone and testosterone: in vivo study in the hairless rat. Skin Pharmacol 1992;5:99-107.

15. Hueber F, Besnard M, Schaefer H, Wepierre J. Percutaneous absorption of estradiol and progesterone in normal and appendage-free skin of the hairless rat: lack of importance of nutritional blood flow. Skin Pharmacol 1994;7:245-56.

16. Lieb LM, Ramachandran C, Egbaria K, Weiner N. Topical delivery enhancement with multilamellar liposomes into pilosebaceous units: I. In vitro evaluation using fluorescent techniques with the hamster ear model. J Invest Dermatol 1992;99:108-13.

17. Lieb LM, Liimatta AP, Bryan RN, Brown BD, Krueger GG. Description of the intrafollicular delivery of large molecular weight molecules to follicles of human scalp Skin *In Vitro*. J Pharm Sci 1997;86(9):1022-9.

18. Lieb LM, Flynn G, Weiner N. Follicular (pilosebaceous unit) deposition and pharmacological behavior of cimetidine as a function of formulation. Pharm Res 1994;11(10):1419-23.

19. Li L, Lishko V, Hoffman RM. Liposomes can specifically target entrapped melanin to hair follicles in histocultured skin. In Vitro Cell Dev Biol 1993;29A:192-4.

20. Vaughan CD. Using solubility parameters in cosmetics formulations. J Soc Cosmet Chem 1985;36:319-33.

21. Nicolau G, Baughman RA, Tonelli A, McWilliams W, Schiltz J, Yacobi A. Deposition of viprostol (a synthetic PGE2 vasodilator) in the skin following topical administration to laboratory animals. Xenobiotica 1987;17:1113-20.

22. Lauer AC, Elder JT, Weiner ND. Evaluation of the hairless rat as a model for in vivo percutaneous absorption. J Pharm Sci 1997 Jan;86(1):13-8.

23. Hueber F, Schaefer H, Wepierre J. Role of transepidermal and transfollicular routs in percutaneous absorption of steroids: in vitro studies on human skin. Skin Pharmacol 1994;7:237-44.

24. Fabin B, Touitou E. Localization of lipophilic molecules penetration rat skin in vivo by quantitative autoradiography. Int J Pharm 1991;74:59-65.

25. Mills OH, Kligman AM. A human model for assaying comedolytic substances. Br J Dermatol 1982;107:543-8.

26. Bojar RA, Cutcliffe AG, Graupe K, Cunliffe WJ, Holland KT. Follicular concentration of azelaic acid after a single topical application. Br J Dermatol 1993;129(4):399-402.

27. Kao J, Helman J. In vitro percutaneous absorption in mouse skin: influence of skin appendages. Tox Appl Pharmacol 1988;94:93-103.

28. Green PG, Hinz RS, Kim A, Szoka FC, Guy RH. Iontophoretic delivery of a series of tripeptides across the skin in vitro. Pharm Res 1991;8:1121-7.

29. Cullander C, Guy RH. Sites of iontophoretic current flow into the skin: identification and characterization with the vibrating probe electrode. J Invest Dermatol 1991;97:55-64.

30. Burnette RR, Ongpipattanakul B. Characterization of the pore transport properties and tissue alteration of excised human skin during iontophoresis. J Pharm Sci 1988;77:132-7.

31. Lauer AC. Percutaneous drug delivery to the hair follicle. In: Bronaugh RL, Maibach HI, editors. Percutaneous Absorption Drug-Cosmetic-Mechanisms-Methodology. 3rd ed. New York: Marcel Dekker, Inc.; 1999. p. 427-49.

32. Barry BW. Drug delivery routs in skin: a novel approach. Adv Drug Deliv Rev 2002;54(Suppl. 1):S31-S40.

33. Grams YY, Alaruikka S, Lashley L, Caussin J, Lynne W, Bouwstra JA. Permeant lipophilicity and vehicle composition influence accumulation of dyes in hair follicles of human skin. Eur J Pharm Sci 2003;18:329-36.

34. Plewig G., Luderschmidt C. Hamster ear model for sebaceous glands. J Invest Dermatol 1977;68:171-6.

35. Illel H, Schaefer H. Transfollicular percutaneous absorption: skin model for quantitative studies. Acta Derm Venerol (Stockh) 1988;68:427-30.

36. Illel H, Schaefer H, Wepierre J, Doucet O. Follicles play an important role in percutaneous absorption. J Pharm Sci 1991;80:424-7.

37. Touitou E, Meidan VM, Horwitz E. Methods for quantitative determination of drug localized in the skin. J Controlled Release 1998;56:7-21.

38. Liao S, Lin J, Dang MT, Zhang H, Kao YH, Fukuchi J, et al. Growth suppression of hamster flank organs by topical application of catechins, alizarin, curcumin, and myristoleic acid. Arch Dermatol Res 2001;293(4):200-5.

39. Weissmann A, Bowden J, Frank B, Horwitz SN, Frost P. Morphometric studies of the hamster flank organ: an improved model to evaluate pharmacologic effects on sebaceous glands. J Invest Dermatol 1984;82(5):522-5.

40. Chen C, Puy LA, Simard J, Li X, Singh SM, Labrie F. Local and systemic reduction by topical finasteride or flutamide of hamster flank organ size and enzyme activity. J Invest Dermatol 1995;105(5):678-82.

41. Matias JR, Orentreich N. The hamster ear sebaceous glands. I. Examination of the regional variation by stripped skin planimetry. J Invest Dermatol 1983;81(1):43-6.

42. Bernard E, Dubios J, Wepierre J. Importance of sebaceous glands in cutaneous penetration of an antiandrogen: target effect of liposomes. J Pharm Sci 1997;86(5):573-8.

43. Touitou E, Levi-Schaffer F, Dayan N, Alhaique F, Riccierr F. Modulation of caffeine skin delivery by carrier design: liposomes versus permeation enhancers. Int J Pharm 1994;103:131-6.

44. Li L, Lishko VK, Margolis LB, Hoffman RM. Product-delivering liposomes specifically target hair follicles in histocultured intact skin. In Vitro Cell Dev Biol 1992;28A:679-81.

45. Rougier A, Lotte C. The stripping technique. In: Shaw VP, Maibach HI, editors. Topical drug bioavailability, bioequivalence and penetration.New York: Plenum Press; 1993. p. 163-82.

46. Balsari AL, Morelli D, Menard S, Veronesi U, Colnaghi MI. Protection against doxorubicin-induced alopecia in rats by liposome-entrapped monoclonal antibodies. FASEB J 1994;8:226-30.

47. Chen C, Li X, Singh SM, Labrie F. Activity of 17beta-(N-alkyl/arylformamido) and 17beta-[(N-alkyl/aryl) alkyl/arylamido]-4-methyl-4-aza-5alpha-androstan-3-ones as 5alpha-reductase inhibitors in the hamster flank organ and ear. J Invest Dermatol 1998;111(2):273-8.

48. Matias JR, Malloy VL, Orentreich N. Synergistic antiandrogenic effects of topical combinations of 5 alpha-reductase and androgen receptor inhibitors in the hamster sebaceous glands. J Invest Dermatol 1988;91(5):429-33.

49. Lu B, Federoff HJ, Wang Y, Goldsmith LA, Scott G. Topical application of viral vectors for epidermal gene transfer. J Invest Dermatol 1997;108:803-8.

50. Niemiec SM, Ramachandran C, Weiner N. Perifollicular transgenic expression of human interlukin-1 receptor antagonist protein following topical application. J Pharm Sci 1997;86:701-8.

51. Li L, Lishko V, Hoffman RM. Liposome targeting of high molecular weight DNA to the hair follicles of histocultured skin: a model for gene therapy of the hair growth processes. In Vitro Cell Dev Biol 1993;29A:258-60.

52. Schreier H, Bouwstra JA. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. J Controlled Release 1994;30:1-15.

53. Plessis JD, Ramachandran C, Weiner N, Muller DG. The Influence of lipid composition and lamellarity of liposomes on the physical stability of liposomes upon storage. Int J Pharm 1996;127:273-8.

54. Musial W, Kubis A. Preliminary assessment of alginic acid as a factor buffering triethanolamine interacting with artificial sebum. Eur J Pharm Biopharm 2003;55:237-40.

55. Bandak S, Ramu A, Barenholz Y, Gabuzon A. Reduced UV-induced degradation of doxorubicin encapsulated in polyethyleneglycol-coated liposomes. Pharm Res 1999;16(6):841-6.

56. Boswell GW, Buell D, Bekersky I. AmBisome (liposomal amphotericine B): a comparative review. J Clin Pharmacol 1998;38(7):583-92.

57. Gabizon A, Goren D, Cohen R, Barenholz Y. Development of liposomal anthracyclines: from basics to clinical applications. J Controlled Release 1998;53:275-9.

58. Manosroi A, Manosroi J. Microencapsulation of human insulin DEAE-dextran complex and the complex in liposomes by the emulsion non-solvent addition method. J Microencapsul 1997;14:761-8.

59. Manosroi A, Wongtrakul P, Manosroi J, Sakai H, Sugawara F, Yuasa M, et al. Characterization of vesicles prepared with various non-ionic surfactants mixed with cholesterol. Colloids Surf B Biointerfaces 2003;30:129-38.

60. Tsuchihashi M, Harashima H, Kiwada H. Development of a pharmacokinetic/pharmacodynamic (PK/PD)-simulation system for doxorubicin in long circulating liposomes in mice using peritoneal P388. J Controlled Release 1999;61(1-2):9-19.

61. Agarwal R, Katare OP, Vyas SP. Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. Int J Pharm 2001;228:43-52.

62. Touitou E, Junginger HE, Weiner N, Nagai T, Mezei M. Liposomes as carriers for topical and transdermal delivery. J Pharm Sci 1994;83(9):1189-203.

63. Varshosaz J, Pardakhty A, Hajhashemi V, Najafabadi AR. Development and physical characterization of sorbitan monoester niosomes for insulin oral delivery. Drug Deliv 2003;10:251-62.

64. Vanlerberghe G, Morancais JL. Niosomes in perspective. S T P Pharma Sciences 1996;6(1):5-11.

65. van Hal DA. Nonionic surfactant vesicles for dermal and transdermal drug delivery. Ph.D. Thesis. Leiden University, The Netherlands; 1994.

66. Lawrence MJ, Chauhan S, Lawrence SM, Barlow DJ. The formation, characterization and stability of non-ionic surfactant vesicles. S T P Pharma Sciences 1996;6(1):49-60.

67. Hofland HEJ. Vesicles as transdermal drug delivery system Ph. D. Thesis. Leiden University, The Netherlands; 1992.

68. Bouwstra JA, van Hal DA, Hofland HEJ, Junginger HE. Preparation and characterization of nonionic surfactant vesicles. Colloids Surf A: Physicochem Eng Aspects 1997;123-124:71-80.

69. Uchegbu IF, Vyas SP. Non-ionic surfactant based vesicles (niosomes) in drug delivery. Int J Pharm 1998;172(33):70.

70. Uchegbu IF, Double JA, Turton JA, Florence AT. Distribution, metabolism and tumoricidal activity of doxorubicin in sorbitan monostearate (Span 60) niosomes in the mouse. Pharm Res 1995;12:1019-24.

71. Bouwstra JA, Hofland HEJ. Niosomes. In: Kreuter J, editor. Colloidal drug delivery systems.New York: Marcel Dekker; 1994. p. 191-217.

72. Yoshioka T, Sternberg B, Florence AT. Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan triester (Span 85). Int J Pharm 1994;105:1-6.

73. Azmin MN, Florence AT, Handjani-vila RM, Stuart JFB, Vanlerberghe G, Whittaker JS. The effect of non-ionic surfactant vesicles (niosome) entrapment on the absorption and distribution of methotrexate in mice. J Pharm Pharmacol 1985;37:237-42.

74. Kiwada H, Niimura H, Kato Y. Tissue distribution and pharmacokinetic evaluation of the targeting efficiency of synthetic alkyl glycoside vesicles. Chem Pharm Bull (Tokyo) 1985;33:2475-82.

75. Assadullahi TP, Hider RCMAJ. Liposome formation from synthetic polyhydroxy lipids. Biochem Biophys Acta 1991;1083:271-6.

76. Baillie AJ, Florence AT, Hume LR, Muirhead GT, Rogerson A. The preparation and properties of niosomes-nonionic surfactant vesicles. J Pharm Pharmacol 1985;37:863-8.

77. Chauhan S, Lawrence MJ. The preparation of polyoxyethylene containing non-ionic surfactant vesicles. J Pharm Pharmacol 1989;41:6P.

78. Montserrat K, Gratzel M, Tundo P. Light-induced charge injection in functional crown ether vesicles. J Am Chem Soc 1980;102:5527-9.

79. Darwish IA, Uchegbu IF. The evaluation of crown ether based niosomes as cation containing and cation sensitive drug delivery systems. Int J Pharm 1997;159:207-13.

80. Uchegbu IF, McCarthy D, Schatzlein A, Florence AT. Phase transitions in aqueous dispersions of the hexadecyl diglycerol ether (C16G2) non-ionic surfactants, cholesterol and cholesteryl poly-24-oxyethylene ether: vesicles, tubules, discomes and micelles. STP Pharm Sci 1996;6(1):33-43.

81. Arunothayanun P, Uchegbu IF, Craig DQM, Turton JA, Florence AT. In vitro/in vivo characterisation of polyhedral niosomes. Int J Pharm 1999;183:57-61.

82. Uchegbu IF, Duncan R. Niosomes containing N-(2-hydroxypropyl) methacrylamide copolymer-doxorubicin (PK1): effect of preparation and choice of surfactant on niosome characteristics and a preliminary study of body distribution. Int J Pharm 1997;155:7-17.

83. Baillie AJ, Coombs GH, Dolan TF, Laurie J. Non-ionic surfactant vesicles, niosomes, as a delivery system for anti-leishmanial drug, sodium stibogluconate. J Pharm Pharmacol 1986;38:502-5.

84. Dimitrijevic D, Lamandin C, Uchegbu IF, Shaw AJ, Florence AT. The effect of monomers and of micellar and vesicular forms of non- ionic surfactants (Solulan C24 and Solulan 16) on Caco-2 cell monolayers. J Pharm Pharmacol 1997;49:611-6.

85. Hofland HEJ, Geest R, Bodde HE, Junginger HE, Bouwstra JA. Estradiol permeation from nonionic surfactant vesicles through human stratum corneum in vitro. Pharm Res 1994;11(5):659-64.

86. Hofland HEJ, Bouwstra JA, Verhoef JC, Buckton G, Chowdry BZ, Ponec M, et al. Safety aspects of non-ionic surfactant vesicles-a toxicity study related to

physicochemical characteristics of non-ionic surfactants. J Pharm Pharmacol 1992;44:287-94.

87. Kiwada H, Niimura H, Fujisaki Y, Yamada S, Kato Y. Application of synthetic alkyl glycoside vesicles as drug carriers. I: preparation and physical properties. Chem Pharm Bull (Tokyo) 1985;33:753-9.

88. Gianasi E, Cociancich F, Uchegbu IF, Florence AT, Duncan R. Pharmaceutical and biological characterisation of a doxorubicin-polymer conjugate (PK1) entrapped in sorbitan monostearate Span 60 niosomes. Int J Pharm 1997;148:139-48.

89. Rowe RC, Sheskey PJ, Weller PJ. Handbook of pharmaceutical excipients. 4th ed. London, UK: The Pharmaceutical Press and American Pharmaceutical Association; 2003.

90. Carafa M, Santucci E, Alhaique F, Coviello T, Murtas E, Riecceiri FM, et al. Preparation and properties of new unilamellar non-ionic/ionic surfactant vesicles. Int J Pharm 1998;160:51-9.

91. Santucci E, Carafa M, Coviello T, Murtas E, Riecceiri FM, Alhaique F, et al. Vesicles from polysorbate 20 and cholesterol- A simple preparation and a characterisation. STP Pharm Sci 1996;6:29-32.

92. Uchegbu IF, Schatzlein A, Vanlerberghe G, Morgatini N, Florence AT. Polyhedral non-ionic surfactant vesicles. J Pharm Pharmacol 1997;49:606-10.

93. New RRC. Liposomes a practical approach. New York: Oxford University Press; 1990.

94. Moser P, Marchandarvier M, Labrude P, Handjani-vila RM, Vigneron C. Haemoglobin niosomes.1. Preparation, physicochemical and oxygen-carrying properties and stability. Pharm Acta Helv 1989;64:192-202.

95. Chopineau J, Lesier S, Ollivon M. Vesicle formation by enzymatic processes. J Am Chem Soc 1994;116:11582-3.

96. Talsma H, Steenbergen MJv, Borchert jCH, Cromelin DJA. A novel technique for the one-step preparation of liposomes and non-ionic surfactant vesicles without the use of organic solvents. Liposome formation in a continuous gas stream: The bubble method. J Pharm Sci 1994;83:276-80.

97. Stafford S, Baillie AJ, Florence AT. Drug effects on the size of chemically defined non-ionic surfactant vesicles. J Pharm Pharmacol 1988;40:26P.

98. Ganesan MG, Weiner ND, Flynn GL, Ho N.F.H. Influence of liposomal drug entrapment on percutaneous absorption. Int J Pharm 1984;20:139-54.

99. Ford JL, Timmins P. The use of thermal analysis in the study of liposomes. In: Ford JL, Timmins P, editors. Pharmaceutical thermal analysis.Chichester: Elis Horwood Limited; 1989. p. 259-78.

100. Grabiel-Madelmont C, Lesieur S, Ollivon M. Characterization of loaded liposomes by size exclusion chromatography. J Biochem Biophys Methods 2003;56:189-217.

101. Rogerson A, Cummings J, Willmott N, Florence AT. The distribution of doxorubicin in mice following administration in niosomes. J Pharm Pharmacol 1988;40:337-42.

102. Archer JS, Chang RJ. Hirsutism and acne in polycystic ovary syndrome. Best Pract Res Clin Obstet Gynecol 2004;18(5):737-54.

103. Beigi A, Sobhi A, Zarrinkoub F. Finasteride versus cyproterone acetate-estrogen regimens in the treatment of hirsutism. Int J Gynaecol Obstet 2004;87:29-33.

104. Ranjan M, Diffley P, Stephen G, Price D, Walton TJ, Newton RP. Comparative study of human steroid 5α-reductase isoforms in prostate and female breast skin tissues: sensitivity to inhibition by finasteride and episteride. Life Sci 2002;71:115-26.

105. Thiboutot D, Harris G, Iles V, Cimis G, Gilliland K, Hagari S. Activity of the type 1 5α–reductase exhibits regional differences in isolated sebaceous glands and whole skin. J Invest Dermatol 1995;105:209-14.

106. Chen W, Zouboulis CC, Orfanos CE. The 5α–reductase system and its inhibitors: recent development and its perspective in treating androgen-dependent skin disorders. Dermatology 1996;193:177-84.

107. Luu-The V. Characterization, expression and immunohistochemical localization of 5α-reductase in human skin. J Invest Dermatol 1994;102:221-6.

108. Harris GS, Kozarich JW. Steroid 5α-reductase inhibitors in androgen-dependent disorders. Curr Opin Chem Biol 1997;1:254-9.

109. Itami S, Kurata, Sonada T, Takayasu S. Characterization of 5α–reductaze in cultured human dermal papilla cells from beard and occipital scalp hair. J Invest Dermatol 1991;96:57-60.

110. Dallob AL, Sadick NS, Unger W, Lipert S, Geissler LA, Gregoire SL, et al. The effect of finasteride, a 5α–reductase inhibitor, on scalp skin testosterone and dihydrotestosterone concentrations in patients with male pattern baldness. J Clin Endocrinol Metab 1994;79:703-6.

111. Rittmaster RS, Uno H, Povar ML, Mellin TN, Loriaux DL. The effect of *N,N*-diethyl-4-methyl-3-oxo-4-aza-5α-androstane-17β carboxamide, a 5α-reductase

inhibitor and antiandrogen, on the development of baldness in the stumptail macaque. J Clin Endocrinol Metab 1987;65:188-93.

112. Bakshi RK, Oatel GF, Rasmusson GH. 7β-Methyl-4-aza-cholestan-3-one (MK386) and related 4-azasteroids as selective inhibitors of human type 1 5α-reductase. J Med Chem 1994;37:3871-4.

113. Martindale: The complete drug reference. 34th ed. London, UK: Pharmaceutical Press; 2005.

114. The Merck index. 13th ed. Whitehouse Station, NJ, USA: Merck and Co., INC.; 2001.

115. Clark's analysis of drugs and poisons. 3rd ed. London: Pharmaceutical press; 2004.

116. Ptacek P, Macek J, Klima J. Determination of finasteride in human plasma by liquid-liquid extraction and high-performance liquid chromatography. J Chromatogr B Biomed Appl 2000;738:305-10.

117. Constanzer ML, Matuszewski BK, Bayne WF. High-performance liquid chromatographic method for the determination of finasteride in human plasma at therapeutic doses. J Chromatogr B Biomed Appl 1991;566:127-34.

118. Carlin JR, Christofalo P, Vandenheuvel WJA. High-performance liquid chromatographic determination of N-(2-methyl-2-propyl)-3-oxo-4-aza-5α-

androst-1-ene-17β–carboxamide, a 4-azastroid, in human plasma from a phase I study. J Chromatogr B Biomed Appl 1988;427:79-91.

119. Syed AA, Amshumali MK. LC determination of finasteride and its application to storage stability studies. J Pharm Biomed Anal 2001;25:1015-9.

120. Takano T, Hata S. High-performance liquid chromatographic determination of finasteride in human plasma using direct injection with column switching. J Chromatogr B Biomed Appl 1996;676:141-6.

121. Carlucci G, Mazzeo P. Finasteride in biological fluids: extraction and separation by a graphitized carbon cartridge and quantification by high-performance liquid chromatography. J Chromatogr B Biomed Appl 1997;693:245-8.

122. Lunn G, Schmuff NR. HPLC methods for pharmaceutical analysis. New York, NY, USA: John Wiley and sons, Inc.; 1997.

123. Matuszewski BK, Constanzer ML, Chavez-Eng CM. Matrix effect in quantitative LC/MS/MS analyses of biological fluids: a method for determination of finasteride in human plasma at picogram per milliliter concentrations. Anal Chem 1998;70:882-9.

124. Guarna A, Danza G, Bartolucci G, Marrucci A, Dini S, Serio M. Synthesis of 5,6,6- [2H3] finasteride and quantitative determination of finasteride in human

137. Radiation safety handbook office of research safety northwest university. 2002. Report No.: HSE 901-14.

138. Radiation safety manual prepared for office of health and safety centers for disease control (CDC). 1999.

139. Technical information. [T-1652-NUC-88-7]. 1988. California, USA, Beckman Instruments, Inc.

140. Scintillation system catalog: LS 6000 series supplies and accessories. [BR-8127A]. 1996. California, USA, Beckman Instruments, Inc.

141. Papahadjopoulos D, Szoka F, inventors; Method of encapsulating biologically active materials in lipid vesicles. United States patent 4 235 871. 1980.

142. MacDonald RC, MacDonald RI, Taleshita K, Subbarao NK, Hu L. Small-volume extrusion apparatus for preparation of large, unilamellar vesicles. Biochem Biophys Acta 1991;1061:297-303.

143. Data presentation and interpretation. In: Allen T, editor. Particle size measurement, powder sampling and particle size measurement. 5th ed. London: Chapman & Hall; 1997. p. 44-111.

144. Particle characterisation. In: Seville JPK, Tuzan U, Clift R, editors. Processing of particulate solids. 1st ed. London: Chapman &Hall; 1997. p. 1-52.

145. Bonina F, Montenegro L. Comparison of different separative techniques in the quantitative determination of active compound enclosed in liposomal systems. Int J Cosmet Sci 1994;16:183-97.

146. Fildes F.J.T., Oliver JE. Interaction of cortisol-21-palmitate with liposomes examined by differential scanning calorimetry. J Pharm Pharmacol 1978;30:337-42.

147. Montenegro L, Panico AM, Bonina F. Quantitative determination of hydrophobic compound entrapped in dipalmitoylphosphatidylcholine liposomes by differential scanning calorimetry. Int J Pharm 1996;138:191-7.

148. Costa P, Lobo JMS. Influence of dissolution medium agitation on release profile of sustained-release tablets. Drug Dev Ind Pharm 2001;27(8):811-7.

149. Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci 2001;13:123-33.

150. Lu DR, Abu-Izza K, Mao F. Nonlinear data fitting for controlled release devices: an integrated computer program. Int J Pharm 1996;129:243-51.

151. Washington C. Drug release from microdisperse systems: a critical review. Int J Pharm 1990;58:1-12.

152. Plessis JD, Ramachandran C, Weiner N, Muller DG. The influence of particle size of liposomes on the deposition of drug into skin. Int J Pharm 1994;103:177-82.

153. Nasseri B, Florence AT. Some properties of extruded non-ionic surfactant micro-tubes. Int J Pharm 2003;254:11-6.

154. Hao Y, Zhao F, Li N, Yang Y, Li K. Studies on a high encapsulation of colchicine by a niosome system. Int J Pharm 2002;244:73-80.

155. Szoka F, Olson F, Heath TVW, Mayhew E, Papahadjopoulos D. Preparation of unilamellar liposomes of intermediate size (0.1-0.2 μm) by a combined of reverse phase evaporation and extrusion through polycarbonate membranes. Biochim Biophys Acta 1980;601:559-71.

156. Jin AJ, Huster D, Gawrisch K, Nossal R. Light scattering characterization of extruded lipid vesicles. Eur Biophys J 1999;28:187-99.

157. Berger N, Sachse A, Bender J, Schubert R, Brandl M. Filter extrusion of liposomes using different devices: comparison of liposome size, encapsulation efficiency, and process characteristics. Int J Pharm 2001;223:55-68.

158. Benatti CR, Tiera MJ, Feitosa E, Olofsson G. Phase behaviour of synthetic amphiphile vesicles investigated by calorimetry and fluorescence methods. Thermochim Acta 1999;328:137-42.

159. Engbert JBFN, Hoekstra D. Vesicle-forming synthetic amphiphiles. Biochim Biophys Acta 1995;1241:323-40.

160. Taylor KMG, Morris RM. Thermal analysis of phase transition behaviour in liposomes. Thermochim Acta 1995;248:289-301.

161. McCauley, Brittain HG. Thermal methods of analysis. In: Brittain HG, editor. Physical characterization of pharmaceutical solids.New York: Marcel Dekker, Inc.; 1995. p. 241.

162. Montenegro L, Panico AM, Ventmiglia A, Bonina VP. In vitro retinoic acid release and skin permeation from different liposome formulations. Int J Pharm 1996;133:89-96.

163. Shah VP, Skelly JP. Practical consideration in developing a quality control (in vitro release) procedure for topical drug products. In: Shah VP, Maibach HI, editors. Topical drug bioavailability, bioequivalence, and penetration.New York: Plenum Press; 1993. p. 107-16.

164. Saarinen-Savolainen P, Jarvinen T, Taipale H, Urtti A. Methods for evaluating drug release from liposomes in sink conditions. Int J Pharm 1997;159:27-33.

165. Manconi M, Sinico C, Valenti D, Loy G, Fadda AM. Niosomes as carriers for tretinoin. I. Preparation and properties. Int J Pharm 2002;234:237.

166. El Maghraby GMM, Williams AC, Barry BW. Skin delivery of oestradiol from deformable and traditional liposomes: mechanistic studies. J Pharm Pharmacol 1999;51:1123-34.

167. Roberts MS, Cross SE. Skin transport. In: Walters KA, editor. Dermatological and transdermal formulations.New York: Marcel Dekker, Inc.; 2002.

168. Fleisher D, Niemiec SM, Oh CK, Ramachandran C, Weiner N. Topical delivery of growth hormon releasing peptide using liposomal systems: an in vitro study using hairless mouse skin. Life Sci 1995;57(13):1293-7.

169. Egbaria K, Weiner N. Liposomes as a topical drug delivery system. Adv Drug Deliv Rev 1990;5:287-300.

170. Tschan T, Steffen H, Supersaxo A. Sebaceous-gland deposition of isotertinoin after topical application: an in vitro study using human facial skin. Skin Pharmacol 1997;10(126):134.

171. Walters KA, Walker M, Olejnik O. Non-ionic surfactants. Effects on hairless mouse skin permeability characteristics. J Pharm Pharmacol 1987;40:525-9.

172. Han I, Kim M, Kim J. Enhanced transfollicular delivery of adriamycin with a liposome and iontophoresis. Exp Dermatol 2004;13(2):86-92.

173. Balsari AL, Morelli D, Menard S, Veronesi U. Protection against doxorubicin-induced alopecia in rats by liposome-entrapped monoclonal antibodies. Res Commun 1994;8:226-31.

174. Toll R, Jacobi U, Richter H, Lademann J, Schaefer H, Blume-Peytavi U. Penetration profile of microspheres in follicular targeting of terminal hair follicles. J Invest Dermatol 2004;123:168-76.

175. Weiner N, Martin F, Riaz M. Liposomes as a drug delivery system. Drug Dev Ind Pharm 1989;15:1523-54.

176. Bernard E, Dubios JL, Wepierre J. Percutaneous absorption of a new antiandrogen included in liposomes or in solution. Int J Pharm 1995;126:235-43.

177. Fang JY, Yu SY, Wu PC, Huang YB, Tsai YH. In vitro skin permeation of estradiol from varios proniosome formulations. Int J Pharm 2001;215:91-9.

178. Fang JY, Hong CT, Chiu WT, Wang YY. Effect of liposomes and niosomes on skin permeation of enoxacin. Int J Pharm 2001;219:61-72.

179. Valjakka-Koskela R, Kirjavainen M, Monkkonen J. Enhancement of percutaneous absorption of naproxen by phospholipids. Int J Pharm 1998;175:225-30.

180. Sarpotdar PP, Zatz JL. Percutaneous absorption enhancement by nonionic surfactants. Drug Dev Ind Pharm 1986;12:1625-47.

دارورسانی هدفمند فیناستراید به فولیکول مو توسط سیستم های لیپوزومی

خلاصه پایا ن نامه

مقدمه: دارورسانی هدفمند فیناستراید به فولیکول مو می تواند غلظت دارو را در پیلوسباسه افزایش دهد و اثرات جانبی سیستمیک آنرا کم نماید.

روشها: نیوزوم و لیپوزوم حاوی دارو به دو روش تشکیل فیلم و تبخیر فاز معکوس تهیه شد و ویژگی آنها از نظراندازه ذره ای، کفایت احتباس، آزادسازی در محیط های فسفات بافرسالین و سبوم مصنوعی، همچنین نفوذ دارواز غشای پوستی در برون تن بررسی گردید. مطالعه درون تنی استقرار فیناستراید نشاندار(^{3}H-finasteride) در پیلوسباسه و دیگر بخشهای گوش هامسترپس از استعمال موضعی وزیکول های حاوی فیناستراید و محلول هیدروالکلی آن به طریقه جدا کردن فولیکولی (scraping) انجام شد. همچنین تاثیر استعمال فرآورده به میزان دو بار در روز به مدت ۴ هفته بر مساحت غدد سباسه گوش و فلانک هامستر به روش تعیین سطح مقطع بافتی پوست مطالعه گردید.

نتایج: سرعت نفوذ فیناستراید ازپوست ناحیه فلانک از محلول هیدروالکلی سریعتر از وزیکولهای حاوی دارو بود (۰/۱۶ $\mu g/cm^2.h$ در برابر $\mu g/cm^2.h$ ۰/۰۵۸-۰/۰۲۵). وزیکولهای حالت مایع باردارطی ۷/۵-۲/۵ساعت ۵۰% از دارو را به درون سبوم مصنوعی آزاد کردند.

استعمال وزیکولهای اکسترودنشده متشکل از آمفی فایل های حالت مایع بریج۹۷ ،DMPC و مخلوط ۱:۱ بریج۷۶ : بریج۹۷ ، منجر به استقرار ۲/۵-۲/۰۵% ازدز استعمال شده در واحد

پیلوسباسه گردید، که بطور بارزی نسبت به وزیکولهای مشابه اکسترودشده (۴۰۰ و۱۰۰ nm) ، وزیکولهای حالت ژل ونیز محلول هیدروالکلی، استقرار بالاتری را موجب گردید (۰/۹۲-۰/۳۵%). وزیکولهای دارای بیشترین استقرارو محلولهای هیدروالکلی فیناسترایدو پروژسترون (کنترل مثبت) بطور مشهودی اندازه غدد سباسه گوش و ناحیه فلانک را در سمت استعمال شده کاهش دادند (۴۸-۳۶% ، $p < ۰/۰۵$)، در حالیکه محلول هیدروالکلی باعث کاهش اندازه این غدد در سمت استعمال شده وسمت استعمال نشده فلانک گردید، که می تواند نشان دهنده اثر سیستمیک آن باشد.

بحث و نتیجه گیری: مطالعات درون تنی استقرار فولیکولی دارو و کاهش اندازه غدد سباسه هامسترپس از تجویز موضعی، می تواند نشان دهنده قابلیت وزیکولهای حالت مایع باردار برای دارورسانی موثرفیناسترایدبه واحد پیلوسباسه باشد.

لغات کلیدی:

فیناستراید، نیوزوم، لیپوزوم، دارورسانی هدفمند، واحد پیلوسباسه، گوش و فلانک هامستر