In Vitro Cell. Dev. Biol. 29A:258–260, April 1993

0883-8364/93 $01.50+0.00

Letter to the Editor

LIPOSOME TARGETING OF HIGH MOLECULAR WEIGHT DNA TO THE HAIR FOLLICLES OF HISTOCULTURED SKIN: A MODEL FOR GENE THERAPY OF THE HAIR GROWTH PROCESSES

Dear Editor:

The histoculture of intact skin on sponge-gel matrices offers many opportunities for tissue engineering and the in vitro study of important processes intrinsic to skin such as hair growth and toxicity to various agents (Li et al., 1991, 1992a, 1992b).

There is an increasing interest in the hair-follicular (appendage) route for delivery of drugs for both trans-dermal drug delivery and delivery of active compounds affecting hair itself. Feldmann and Maibach (1967) and Maibach (1971) noted that in vitro absorption of various compounds tested depended upon the regional difference in the morphology of human skin used. Absorption was increased in areas of increased follicular density and size, e.g. forehead and scalp. Recently, Illel et al. (1991) reported that the in vitro percutaneous steady-state flux for representative penetrants was 2–4 times higher in normal appendage-containing, hairless rat skin (follicular density 10–200/cm^2) relative to their appendage-free (follicle-free) skin. Other reports in the literature also suggested significant amount of penetration via the pilosebaceous apparatus (Bidmon et al., 1990; Rutherford et al., 1969; Suzuki et al., 1978).

Liposomes have been widely and successfully used as delivery systems to transport macromolecular substances into the cell which can not normally cross the plasma membrane (Gregoriadis 1984, 1988; Egbaria and Weiner, 1990). We have recently reported that calcein-dye entrapped liposomes specifically targeted hair follicles in histocultured intact skin (Li et al., 1992d). Our results were strongly confirmed by studies by Lieb et al. (1992) demonstrating that liposomal formulations were more efficient than any other vehicle tested for delivery of carboxyfluorescein through the follicular route into the pilosebaceous units with the hamster ear model. We then developed liposome-mediated targeted delivery of melanin into hair follicles and the hair shaft itself in histocultured white-haired mouse skin (Li et al., 1993). These studies have many ramifications including targeted hair growth modification and trans-follicular transdermal delivery systems.

A number of years ago we developed the technique of entrapping DNA in liposomes (Hoffman et al., 1978). We have utilized DNA liposomes in the studies reported here to target high molecular weight DNA to the hair follicle itself as a model of gene therapy of the hair growth processes.

A one kb DNA fragment was isolated from a mouse genomic DNA library and purified from low melting point agarose with the Magic PDR DNA Purification Kit (Promega, Madison, WI). 50 ng of DNA was labeled with [^{35}S]dATP (Dupont) with the Random Primer DNA Labeling Kit (BioRad, Richmond, VA). The specific activity of the labeled DNA with ^{35}S-dATP was 2.6×10^{10} cpm/μg.

Liposomes were prepared by freezing and thawing. 20 mg of egg phosphatrolycholine (PC) was rotary evaporated with a vacuum drier from a chloroform solution to form a thin film on the walls of a 5 ml round-bottomed flask for 1 hour. The dried thin film lipid was suspended in 0.5 ml phosphate buffered saline (PBS, pH 7.4) on a vortex mixer and then sonicated with a Branson probe-type sonicator fitted with a microtip at power level 3, for 8 minutes. The 0.5 ml of [^{35}S]dATP-labeled DNA solution was entrapped with the above suspension by extensive vortexing for 1 minute followed 3 times by freezing and thawing. Liposomes were separated from the non-entrapped [^{35}S]dATP by gel-filtration on a Sepharose 4B column diluted with PBS. 50 μl of Calcein (10 mg/ml) was added into the above solution in order to mark the liposomes during the separation. The specific activity of entrapped DNA labeled with [^{35}S]dATP was 2.5×10^{10} cpm/μl measured by liquid scintillation counting.

Pieces of outbred white-haired-mouse skin (approximately $2 \times 5 \times 2$ mm) derived from 1–5 week-old animals were harvested under a dissection microscope and then histocultured on collagen-gel-supported sponges as described earlier (Li et al., 1991). Liposome interaction with the skin was initiated after 24 hours of histoculture. Mouse skin histocultures were then incubated subsequently for 44 hours with liposomes. A solution of naked-[^{35}S]DNA at the same concentration was used in the liposome preparation to serve as the control and was also incubated with skin histocultures.

The skin histocultures were then fixed with formalin and processed through the standard procedure of dehydration, paraffin-embedding and sectioning. The slides then were prepared for autoradiography by the method which we have used before (Li et al., 1991).

The histological autoradiogram of Figure 1 shows [^{35}S]DNA-labeled hair and follicle cells in histocultured skin after the histocultured skin was incubated with the DNA liposomes for 44 hours. The radio-labeled cells have grains over them that appear green due to reflection of the polarized light of the microscope. High radioactive labeling by the [^{35}S]DNA in the cell membranes and cell cytoplasm as well as in the cell nucleus can be noted in Figure 1. This suggests that the liposomes have delivered the DNA across the cell membrane and the DNA is eventually transported through the cytoplasm to the nucleus.

When the histocultured skin was treated with naked-[^{35}S]DNA there were only a few radioactive labeled cells (data not shown). For further comparison, we have calculated from the autoradiograms the percent of labeled follicles per 20× field and the percent of labeled cells per follicle in the areas of maximum labeling. As can be seen in Table 1, both the percent of labeled follicles per 20×

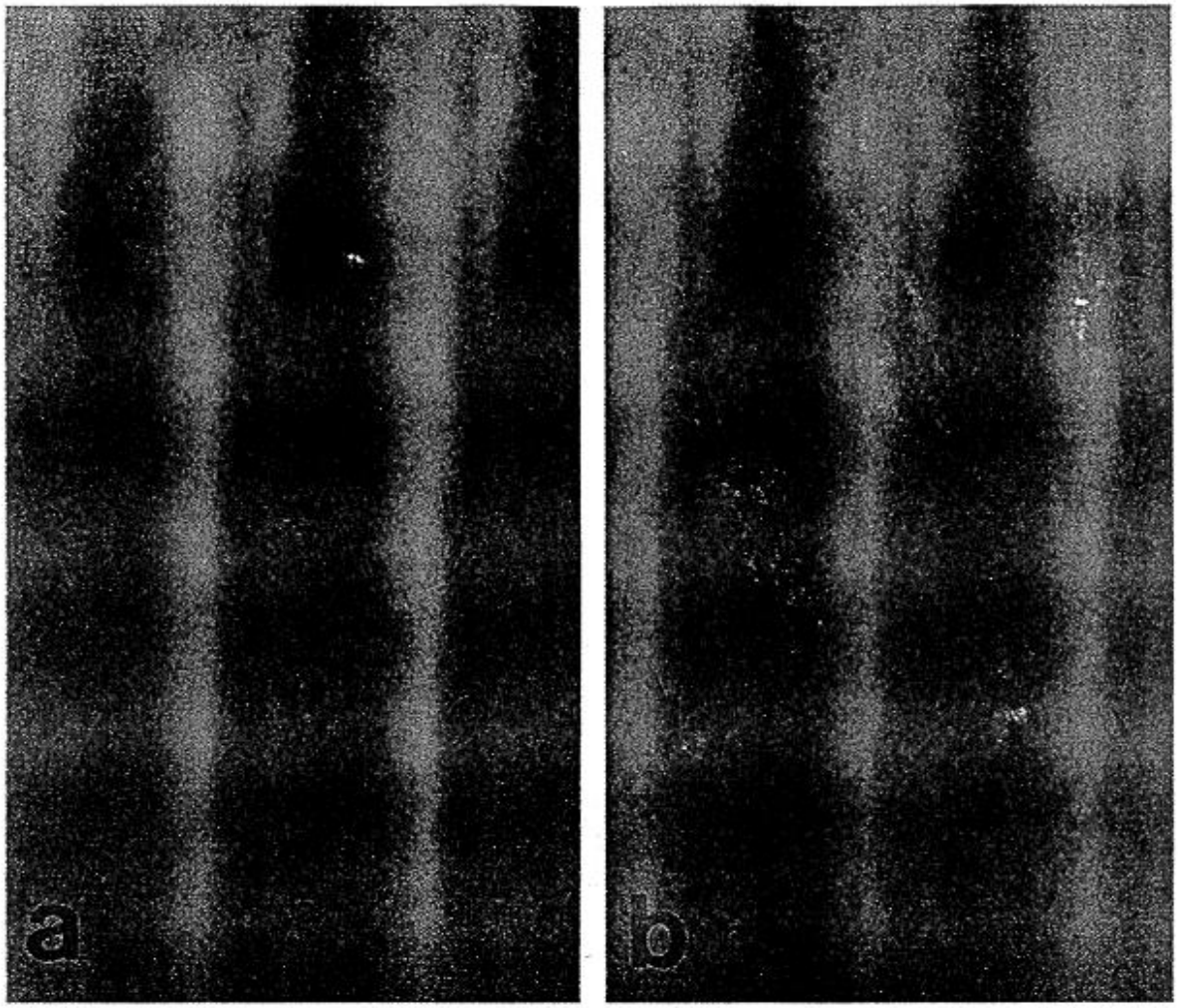

FIG. 1. Histological autoradiogram of histocultured skin after incubation with liposomes entrapped with [^{35}S]DNA for 44 hours. *Note* the high radioactive labeling by the [^{35}S]DNA of hair follicle cells which can be seen in the cell membrane and cell cytoplasm as well as in the cell nucleus. The radiolabeled cells have grains over them that appear green due to reflection of the polarized light of the microscope. Epi-polarization microscopy. Magnification ×500.

microscope field and the percent of labeled cells per follicle in liposome-[^{35}S]DNA treated skin histocultures are significantly higher than in naked-[^{35}S]DNA treated histocultures.

TABLE 1

LIPOSOME TRANSFER OF [^{35}S]DNA TO HAIR FOLLICLES OF HISTOCULTURED SKIN[a]

	Lipo-[^{35}S]DNA	Naked-[^{35}S]DNA	
Percent of labeled follicles per 20× field	37.50 (6/16)	5.41 (2/37)	$P < 0.05$
Percent of labeled cells per follicle	51.06 (24/47)	9.30 (4/43)	$P < 0.005$

[a] [^{35}S]DNA was entrapped in PC liposomes as described in the text. The liposomes were incubated with the skin histocultures as described in the text. Naked [^{35}S]DNA was used as a control. Follicular [^{35}S]DNA was analyzed by histological autoradiography as described in the text in the areas of maximum labeling.

Our results thus demonstrate that liposomes can target DNA into the hair follicle. This gives rise to the possibility of liposomes delivering genes to the hair follicle that could alter hair pigment (Bouchard et al., 1989) and the growth of hair itself. Liposomes have been utilized in experiments which are designed toward development of gene therapy of cancer and other diseases (Zhou et al., 1992). The distinct advantage of liposome-based gene therapy of the hair-growth process is that liposomes seem to actually specifically target the hair follicle.

ACKNOWLEDGEMENTS

This work was supported in part by U.S. National Cancer Institute Small Business Innovation Research Grants 1R43CA53995-01A1 and 1R43CA57072-01. We thank Ms. Polly Jayne Pomeroy for expert word processing of the manuscript.

REFERENCES

Bidmon, H. J.; Pitts, J. D.; Solomon, H. F., et al. Estradiol distribution and penetration in rat skin after topical application studied by high resolution autoradiography. Histochemistry 95:43–54; 1990.

Bouchard, B.; Fuller, B. B.; Setaluri, V., et al. Induction of pigmentation in mouse fibroblasts by expression of human tyrosinase cDNA. J. Exp. Med. 169:2029–2042; 1989.

Feldmann, R. J.; Maibach, H. I. Regional variation in percutaneous penetration of ^{14}[C] cortisol in man. J. Invest. Dermatol. 48:181–183; 1967.

Gregoriadis, G., ed. Liposome technology, volume II. Incorporation of drugs, proteins, and genetic materials. London, Florida: CRC Press, Inc.; 1984.

Gregoriadis, G., ed. Liposomes as drug carriers. Recent trends and progress. London: John Wiley and Sons; 1988.

Haugland, R. P., ed. Liposome technology, volume II. Incorporation of drugs, proteins and genetic materials. London: CRC Press, Inc.; 1984.

Hoffman, R. M.; Margolis, L. B.; Bergelson, L. D. Binding and entrapment of high molecular weight DNA by lecithin liposomes. FEBS Letters 93:365–368; 1978.

Hoffman, R. M. Three-dimensional histoculture: origins and applications in cancer research. Cancer Cells 3:86–92; 1991.

Illel, B.; Schaefer, H.; Wepierre, J., et al. Follicles play an important role in percutaneous absorption. J. Pharm. Sci. 80:424–427; 1991.

Illel, B.; Schaefer, H. Transfollicular percutaneous absorption: a model for quantitative studies. Acta. Dermatol. Venerol. 68:427–430; 1988.

Li, L.; Margolis, L. B.; Hoffman, R. M. Skin toxicity determined *in vitro* by three-dimensional, native-state histoculture. Proc. Natl. Acad. Sci. USA 88:1908–1912; 1991.

Li, L.; Paus, R.; Margolis, L. B., et al. Hair growth in vitro from histocultured mouse skin. In Vitro Cell. Dev. Biol. 28A:479–481; 1992a.

Li, L.; Margolis, L. B.; Paus, R., et al. Hair shaft elongation, follicle growth, and spontaneous regression in long-term, sponge-gel supported histoculture of human scalp skin. Proc. Natl. Acad. Sci. USA 89:8764–8768; 1992b.

Li, L.; Paus, R.; Slominski, A., et al. Skin histoculture assay for studying the hair cycle. In Vitro Cell. Dev. Biol. 28A:695–698; 1992c.

Li, L.; Margolis, L. B.; Lishko, V. K., et al. Product-delivery liposomes specifically target hair follicles in histocultured intact skin. In Vitro Cell. Dev. Biol. 28A:679–681; 1992d.

Li, L.; Lishko, V.; Hoffman, R. M. Liposomes can specifically target entrapped melanin to hair follicles in histocultured skin. In Vitro Cell. Dev. Biol. 29A:192–194; 1993.

Lieb, L. M.; Ramachandran, C.; Egbaria, K., et al. Topical delivery enhancement with multilaminar liposomes into pilosebaceous units: in vitro evaluation using fluorescent techniques with the hamster ear model. J. Invest. Dermatol. 99:108–113; 1992.

Maibach, H. I.; Feldmann, R. J.; Milby, T. H., et al. Regional variation in percutaneous penetration in man. Arch. Environ. Health 23:208–211; 1971.

Rutherford, T.; Black, J. G. The use of autoradiography to study the localization of germicides in skin. Br. J. Dermatol. 81:75–87; 1969.

Suzuki, M.; Asaba, K.; Komatsu, H., et al. Autoradiography study on percutaneous absorption of several oils useful for cosmetics. J. Soc. Cosmet. Chem. 29:265–282; 1978.

Zhou, F.; Rouse, B. T.; Huang, L. Prolonged survival of thymoma-bearing mice after vaccination with a soluble protein antigen entrapped in liposomes: a model study. Cancer Res. 52:6287–6291; 1992.

Lingna Li
Valeryi Lishko

Robert M. Hoffman

AntiCancer, Inc.
5325 Metro Street
San Diego, California 92110 (L. L., V. L., R. M. H.) and
Laboratory of Cancer Biology
Department of Pediatrics, 0609F
University of California, San Diego
La Jolla, California 92093 (R. M. H.)

(Received 17 December 1992)