Journal of Drug Targeting, August–September 2008; 16(7–8): 591–595

informa
healthcare

SHORT COMMUNICATION

Using PEGylated nano-liposomes to target tissue invaded by a foreign body

AVI SCHROEDER[1,2,*], ALEX SIGAL[1,*], KEREN TURJEMAN[1,*], & YECHEZKEL BARENHOLZ[1]

[1]*Laboratory of Liposome and Membrane Research, Department of Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel, and* [2]*Department of Chemical Engineering, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel*

(*Received 24 March 2008; revised 1 May 2008; accepted 13 May 2008*)

Abstract
The ability of nano (~100 nm) sterically stabilized liposomes (nSSLs) to target tissue invaded by a foreign body was demonstrated. Radioactively labeled nSSL remote loaded with the anti-inflammatory drug methylprednisolone hemisuccinate (MPS), to form nSSL–MPS, were injected i.v. to mice that, 2 or 3 weeks earlier, had either a thorn or a needle implanted in a hind leg. Twenty-four hours post-nSSL–MPS injection, animals were sacrificed, and the level of liposomes in the vicinity of the foreign body, in comparison to the tissue in the contralateral (normal) leg, was measured. The level of liposomes in the tissue surrounding the foreign body was twice as high as the level of liposomes found in the normal leg. Furthermore, the level of liposomes in the normal leg was similar to the level of liposomes measured in the legs of control animals that did not have an implanted foreign body and were treated with nSSL–MPS. The implications of these findings and the clinical applications of liposomal targeting are discussed.

Keywords: *Sterically stabilized liposome, passive targeting, foreign body injury, shrapnel, inflammation*

Abbreviations: *MPS, methylprednisolone hemisuccinate; nSSL, nano-sterically stabilized liposome; SUV, small unilamellar vesicle; PEG, polyethylene glycol*

Introduction

The anti-cancer drug Doxil was the first nano-drug delivery system to be approved by the FDA (Barenholz 2001, 2007). Doxil is a formulation of ~100 nm PEGylated liposomes, also referred to as nano-sterically stabilized liposomes (nSSLs), remote loaded with doxorubicin by means of an ammonium sulfate gradient (Haran et al. 1993). Since then, many other liposomal drugs, varying in size, structure, and lipid composition of the liposome, and with different therapeutic aims, have been approved. The rationale behind the development of nSSL drug delivery systems is to improve drug pharmacokinetics and biodistribution (Barenholz 2001; Torchilin 2005), utilizing the compromised vasculature in these diseased tissues (Dvorak et al. 1988), as shown in cases of cancer (Gabizon et al. 1994, 1997), inflammatory conditions (Crommelin et al. 1999; Metselaar et al. 2004; Avnir et al. 2008), and infectious diseases (Storm et al. 1998).

In this study, we sought to utilize these targeting abilities to deliver drugs, such as the anti-inflammatory steroid methylprednisolone hemisuccinate (MPS), to tissue invaded by a foreign body. Examples of foreign body injuries are those resulting from explosions (e.g., shrapnel), work accidents, etc. In many cases, it is

Correspondence: Y. Barenholz, Laboratory of Liposome and Membrane Research, Department of Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel. Tel: 972 2 6757615. Fax: 972 2 6757499. E-mail: yb@cc.huji.ac.il.
*These authors equally contributed to this work.

ISSN 1061-186X print/ISSN 1029-2330 online
DOI: 10.1080/10611860802228939

contraindicated to attempt to surgically remove these foreign bodies, as such a procedure may injure vital organs. In most cases, the foreign body becomes a constant source of pain and inflammation (Chaudhri et al. 1994; McKenzie and Tiernan 2004; Miller et al. 2004; Peyser et al. 2006).

We approached the study of this problem by implanting a foreign body into the hind leg of mice. After 2 or 3 weeks, radioactively labeled nSSL–MPS was injected i.v. After 24 h, enabling liposomes to passively target the tissue invaded by the foreign body (Gabizon et al. 1997), tissues surrounding the implant as well as a number of organs were removed, and their radioactivity was quantified.

Materials and methods

Preparation of ^{3}H-labeled nSSL–MPS

Nano-sterically stabilized liposomes composed of fully hydrogenated soybean phosphatidylcholine, Tm 53°C, Mw 750, having an iodine value of 3.0 (Lipoid, Ludwigshafen, Germany), 51 mol%; methoxy 2-kDa polyethylene glycol–distearoyl phosphoethanolamine (^{2000}PEG–DSPE), Mw 2774 (Genzyme, Liestal, Switzerland), 5 mol%; cholesterol (Sigma, St Louis, MO, USA), 43.56 mol%; and a trace amount of ^{3}H-labeled cholesteryl hexadecyl ether (Perkin-Elmer, Boston, MA), 0.44 mol% (0.5 μCi/μmol phospholipid) were remote loaded with the highly potent anti-inflammatory steroid MPS, Mw 496.53, (Pharmacia, Puurs, Belgium), as previously described (Schroeder et al. 2007; Avnir et al. 2008).

Final MPS and phospholipid levels were determined by HPLC (Schroeder et al. 2007) and the modified Bartlett method (Shmeeda et al. 2003), respectively, and indicated a drug-to-phospholipid mole ratio of ~0.33.

In vivo *model*

All *in vivo* experiments followed strict ethical guidelines dictated by the Ethics Committee and the Institutional Animal Care and Use Committee of the Hebrew University—Hadassah Medical Center, Jerusalem, Israel.

Eight-week-old female SJL mice of similar weight were divided into three test groups (five animals per group): (a) thorn-implanted, (b) needle-implanted, and (c) not implanted (control). Animals of groups (a) and (b) were anesthetized using a ketamine–xylazine solution injected i.p., and the fur over the left hind leg was removed using a hair-removal cream (Depicare, Careline, Yeruham, Israel). Then either a thorn (cut 1 cm from the tip, measuring ~1.4 mm diameter at the base) from a *Lycium europaeum* bush, or a 19G injection needle tip (1.1 mm in diameter, cut 1 cm from the tip), each sterilized in ethanol, was fully inserted into the upper muscle of the leg, where they remained for 2 or 3 weeks. During this period, the animals did not show any stress-related symptoms, behavioral changes, or weight loss. Two weeks after implanting the thorn and 3 weeks after implanting the needle, either 100 μl [to group (b)] or 200 μl [to groups (a) and (c)] of the radioactively labeled nSSL dispersion (8 mM phospholipids) was injected i.v., and 24 h later mice were sacrificed by injecting i.p. 200 μl of a 4%wt chloral-hydrate (Sigma) in 0.9%wt saline (TEVA, Ashdod, Israel). Kidneys, liver, spleen, heart, and lungs, as well as the tissue surrounding the implants, and the tissue from the opposite, healthy leg were extracted and quantified for the liposomal marker (^{3}H), as described below.

Analytical procedures

Liposome size distribution analysis. Liposome size distribution was measured by dynamic light scattering using an ALV-NIBS/HPPS particle sizer equipped with an ALV-5000/EPP multiple digital correlator, at a scattering angle of 173° (ALV, Langen, Germany). Liposome diameter was found to be ~84 nm.

MPS quantification. The level of MPS in ^{3}H-labeled nSSL was determined by immersing samples in a chloroform–sample–methanol (1:1:1 by vol) solution, which was vortexed for 1 min and then centrifuged, using a desk centrifuge, to form two phases. The upper, aqueous phase, which contains >99% of the MPS (verified by using an internal standard of a similar non-liposomal steroid, hydrocortisone, at a known concentration), was separated from the chloroform-rich lower phase, which contains the lipids. The drug level was then determined by HPLC as previously described (Schroeder et al. 2007). MPS extraction from tissue and plasma and quantification were described in detail by Smith et al. (Smith 1979) and Barenholz and coworkers (Avnir et al. 2008)

Liposome biodistribution. After organs and tissue were extracted and homogenized (Polytron, Kinematica, Germany) in 5 ml 30% Triton X-100, they were kept for 72 h at 37°C, to properly solubilize the lipid in the detergent. Then, 15 ml of a liquid scintillation cocktail (Opti-Fluor, Packard Bioscience, Meriden, CT, USA) was added to the samples and left to incubate overnight at RT. The radioactivity level was measured using a β-ray liquid scintillation counter (Betamatic, Kontron) and then the radioactive level was normalized according to tissue weight.

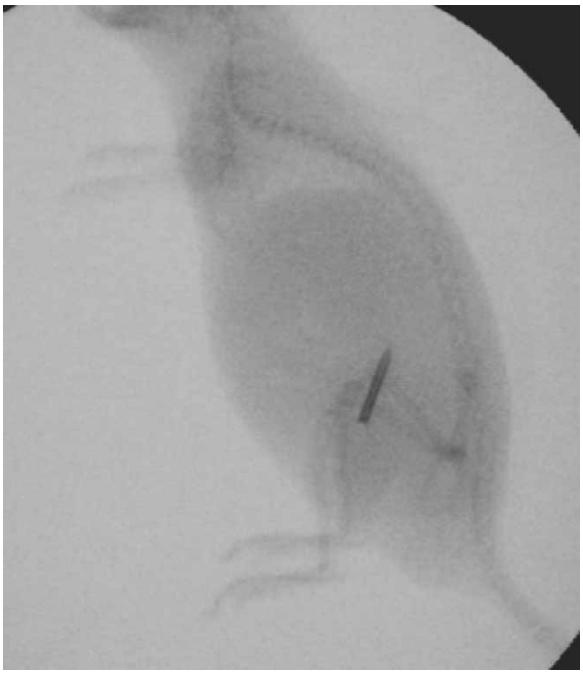

Figure 1. An X-ray image of an implanted needle in the upper muscle of the hind leg of an SJL mouse.

Results and discussion

Injuries in which foreign bodies penetrate organs or tissues are prevalent in, among others, teenagers working in garden maintenance and supply stores (Dunn et al. 1998), construction workers (Driscoll and Hanson 1997; MMWR 2007), civilians, and soldiers injured in war zones (Gasparovic et al. 2004; Gawande 2004; McKenzie and Tiernan 2004; Peleg et al. 2006). Due to the nature of these injuries, they are not confined to specific body organs or tissue. Therefore, we sought to develop a treatment modality that can target the tissue invaded by foreign bodies in multiple locations, irrespective of their composition, by utilizing the exceptional targeting abilities of nSSL.

Herein, we describe a study on the ability of nSSL, loaded with the anti-inflammatory drug MPS, to target tissue in which an implanted foreign body leads to local inflammation. The nSSL are expected to accumulate at the inflamed site still loaded with a sufficient level of drug, which then can be released in a controlled manner, for example, by means of manipulating the liposome formulation features and/or by imposed chemical or physical means, such as focused ultrasound (Barenholz and Crommelin 1994; Barenholz 2001; Schroeder et al. 2007).

Foreign body injuries were simulated by implanting either a thorn or the tip of an injection needle into the muscle of a hind leg of SJL mice (Figure 1). Two or three weeks later, radioactively labeled nSSL loaded with MPS were introduced i.v., and the level of liposomes that targeted the vicinity of the foreign body were quantified.

Measuring the level of nSSL–MPS in the leg with an implanted needle or thorn showed (Table I) that, as a percentage of the administered dose, nearly twice the amount of liposomes reached the tissue surrounding the implanted foreign body ($0.42\% \pm 0.14$ and $0.44\% \pm 0.07$ for implanted thorn and needle, respectively), in comparison with the other (normal) leg ($0.22\% \pm 0.07$ and $0.19\% \pm 0.05$ for implanted thorn and needle, respectively). This suggests that a similar preferential effect occurs in both cases, irrespective of the nature of the implanted foreign body (thorn or needle), most likely due to inflammation in the tissue surrounding the foreign body. Furthermore, the drug level (calculated from the radioactive level) in the leg with an implanted thorn was 1.09 ± 0.36 ng MPS in comparison to 0.57 ± 0.19 ng MPS in the contralateral (normal) leg, and 0.58 ± 0.10 ng MPS in the leg with an implanted needle in comparison to 0.26 ± 0.07 ng MPS in the contralateral (normal) leg. Taking into account that the actual dose administered to animals with an implanted thorn was twice as high as the dose administered to animals with an implanted needle

Table I. nSSL levels in animals with different implanted foreign bodies.

	Percentage of total injected dose		
Model animal	Thorn	Needle	Control (mice with no implant)
Leg with implant	0.42 ± 0.14	0.44 ± 0.07	0.24 ± 0.04
Normal leg	0.22 ± 0.07	0.19 ± 0.05	0.23 ± 0.10
Heart	0.32 ± 0.08	0.33 ± 0.09	0.42 ± 0.05
Liver	0.70 ± 0.19	0.57 ± 0.12	0.73 ± 0.14
Spleen	0.77 ± 0.07	0.59 ± 0.12	1.8 ± 0.16
Kidney	0.91 ± 0.14	0.91 ± 0.18	1.54 ± 0.21
Lungs	0.49 ± 0.05	0.44 ± 0.09	0.63 ± 0.16

Using *t*-test, a statistically significant difference was found between the leg with an implanted foreign body and the legs of animals in the control group or normal legs of animals with an implant ($p < 0.02$ for the thorn and $p < 0.01$ for the needle). Furthermore, normal legs (contralateral to the legs with the implant) of animals with an implanted foreign body had nSSL similar to those found in legs of the control group. In other organs, a statistically significant lower nSSL level was found in the spleen and kidneys ($p < 0.001$ and 0.005, respectively) of animals with an implanted foreign body in comparison with animals in the control group.

(0.26 and 0.13 mg MPS in thorn and needle legs, respectively, see Materials and methods), the actual drug levels in tissue seem to be comparable. Suggesting that the ratio of drug in the leg with the implanted foreign body to that in the other healthy leg is maintained, irrespective of the administered dose (~2-fold increase in the leg with the implant), and that the actual drug level in tissue is dictated by the overall administered dose.

The levels of nSSL found in the healthy legs of animals with an implanted foreign body (Table I) were similar to the nSSL levels found in both legs of the control group, which had no implanted foreign body but was administered nSSL–MPS (0.22% ± 0.07 in the *healthy* legs of animals with an implanted thorn, 0.19% ± 0.05 in the *healthy* legs of animals with an implanted needle, and either 0.24% ± 0.04 or 0.23% ± 0.10 in the legs of the control group). These data, together with the fact that the levels of nSSL in the heart, liver, kidneys, spleen, and lungs of animals with an implanted foreign body or in the control group are unaffected by the presence of a foreign body (Table I), suggest that inflammation-related passive targeting is the main mechanism by which liposomes are delivered to tissue in the vicinity of the foreign body.

Despite the elevated concentration of nSSL found in tissue with an implanted foreign body, and the relatively high level of nSSL in this tissue in respect to the level of nSSL recovered from the pooled organs (10.8% ± 1.5 of the total nSSL recovered from the legs, heart, liver, kidney, spleen, and lungs of animals with an implanted thorn; and 18.7% ± 8.4 of the nSSL recovered from similar organs of animals with an implanted needle), the overall recovery of nSSL from all organs was slightly lower than expected, most likely due to the method of recovery and to the presence of nSSL in other organs (such as the skin) or in circulation, which were not in the scope of this study (Allen et al. 1991; Gabizon et al. 2003); however, this aspect requires further investigation.

Conclusions

This pilot study tested the ability to target tissue damaged by a foreign body, simulated by either an implanted thorn or a needle, with nSSL loaded with a highly potent anti-inflammatory drug. Twenty-four hours after administering nSSL i.v., their level in the vicinity of the implanted foreign body was found to be twice as high as their level in the contralateral (normal) leg or in the legs of control animals. While the actual level of nSSL in the target site is dictated by the total administered dose, the ratio between the level of nSSL in tissue with an implanted foreign body and the contralateral healthy tissue seems to be constant and independent of the nature of the implant. We propose that this approach may be utilized for targeting drugs, such as anti-inflammatories, to tissue damaged by foreign bodies; furthermore, such an approach may prove to be useful for the targeting of drugs to the vicinity of implanted medical devices, in order to achieve a localized therapeutic effect.

Taking into consideration the results presented herein, further preclinical studies of the mechanism of targeting and the extent of such an approach for targeting invading foreign bodies by nSSL are warranted.

Acknowledgments

Avi Schroeder thanks the Barenholz Fund for its financial support; Mr Sigmund Geller is acknowledged with pleasure for his help in editing this manuscript.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Allen TM, Hansen C, et al. 1991. Liposomes containing synthetic lipid derivatives of poly(ethylene glycol) show prolonged circulation half-lives *in vivo*. Biochim Biophys Acta 1066(1):29–36.

Avnir Y, Ulmansky R, et al. 2008. Amphipathic weak acid glucocorticoid prodrugs remote-loaded into sterically stabilized nanoliposomes evaluated in arthritic rats and in a beagle dog: A novel approach to treating autoimmune arthritis. Arthritis Rheum 58(1):119–129.

Barenholz Y. 2001. Liposome application: Problems and prospects. Curr Opin Colloid Interface Sci 6(1):66.

Barenholz Y. 2007. Amphipathic weak base loading into preformed liposomes having a transmembrane ammonium ion gradient: From the bench to approved doxil. London: Taylor & Francis.

Barenholz Y, Crommelin DJA. 1994. Liposomes as pharmaceutical dosage forms. New York: Marcel Dekker.

Chaudhri KA, Choudhury AR, et al. 1994. Penetrating craniocerebral shrapnel injuries during "operation desert storm": Early results of a conservative surgical treatment. Acta Neurochirurgica 126(2–4):120–123.

Crommelin DJA, van Rensen AJ, et al. 1999. Liposomes in autoimmune diseases: Selected applications in immunotherapy and inflammation detection. J Control Release 62(1/2):245–251.

Driscoll T, Hanson M. 1997. Work-related injuries in trade apprentices. Aust NZJ Public Health 21(7):767–772.

Dunn KA, Runyan CW, et al. 1998. Teens at work: A statewide study of jobs, hazards, and injuries. J Adolesc Health 22(1):19–25.

Dvorak HF, Nagy JA, et al. 1988. Identification and characterization of the blood vessels of solid tumors that are leaky to circulating macromolecules. Am J Pathol 133(1):95–109.

Gabizon A, Catane R, et al. 1994. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer Res 54(4):987–992.

Gabizon A, Goren D, et al. 1997. Long-circulating liposomes for drug delivery in cancer therapy: A review of biodistribution studies in tumor-bearing animals. Adv Drug Deliv Rev 24(2):337–344.

Gabizon A, Shmeeda H, et al. 2003. Pharmacokinetics of PEGylated liposomal doxorubicin. Clin Pharmacokinetics 42(5):419–436.

Gasparovic H, Stern-Padovan R, et al. 2004. Intracardiac shrapnel in a polytraumatized child. Anal Thoracic Surg 77(3):1083–1085.

Gawande A. 2004. Casualties of war—military care for the wounded from iraq and afghanistan. NEJM 351(24):2471–2475.

Haran G, Cohen R, et al. 1993. Transmembrane ammonium sulfate gradients in liposomes produce efficient and stable entrapment of amphipathic weak bases. Biochim Biophys Acta, Biomem 1151(2):201.

MMWR. 2007. Nail-gun injuries treated in emergency departments CDC Reports 56. 14, p 329–332.

McKenzie J, Tiernan E. 2004. Hidden shrapnel injury. Emerg Med J 21:264–265.

Metselaar JM, van den Berg WB, et al. 2004. Liposomal targeting of glucocorticoids to synovial lining cells strongly increases therapeutic benefit in collagen type II arthritis. Ann Rheum Dis 63:348–353.

Miller JT, Scheidler MG, et al. 2004. Cystoscopic removal of a large-caliber bullet from the left ureter: A case report of missile igration after a gunshot wound. J Traum Inj Infec Crit Care 56(5):1141–1143.

Peleg K, Rivkind A, et al. 2006. Does body armor protect from firearm injuries? J Am College Surgeons 202(4):643–648.

Peyser A, Khoury A, et al. 2006. Shrapnel management. J Am Acad Orthop Surg 14(10):S66–S70.

Schroeder A, Avnir Y, et al. 2007. Controlling liposomal drug release with low frequency ultrasound: Mechanism and feasibility. Langmuir 23(7):4019–4025.

Shmeeda H, Even-Chen S, et al. 2003. Enzymatic assays for quality control and pharmacokinetics of liposome formulations: Comparison with nonenzymatic conventional methodologies. Methods Enzymol 367(Liposomes):272–292.

Smith MD. 1979. High-performance liquid chromatographic determination of hydrocortisone and methylprednisolone and their hemisuccinate esters in human serum. J Chromatogr 164(2):129–137.

Storm G, Marian TTK, et al. 1998. Doxorubicin entrapped in sterically stabilized liposomes: Effect of bacterial blood clearance capacity of the mononuclear phagocyte system. Clin Cancer Res 3:111–115.

Torchilin VP. 2005. Recent advances with liposomes as pharmaceutical carriers. Nature Rev Drug Discov 4:145–160.