

Collegiate Inventors Competition®

Sample Patent/Literature Search

APPENDIX

Patent Search

A search on the USPTO database yielded the following results. Apart from patents 6,974,699 and 6,472,365 titled 'Pharmaceuticals and assays using enzyme subunits', the other patents discussed below are not within the direct scope of triggered liposomal release but were included for comprehensiveness.

1. Search terms: "Bacteria" AND "Liposomes"

No relevant results found

2. Search terms: "Lipase" AND "Liposomes"

6,974,699 Pharmaceuticals and assays using enzyme subunits
6,472,365 Pharmaceuticals and assays using enzyme subunits

These patents describe an approach using the reconstituted activity of Phospholipase C (alpha-toxin) from *Clostridium Perfringens* to target the *in vivo* release of liposomal contents. Briefly, alpha-toxin is expressed and purified as two separate fragments. The first fragment is conjugated to an antibody targeted to a relevant disease marker. Following administration of the first fragment, a subsequent injection of the second fragment then reconstitutes alpha-toxin at the disease site. The third and final step is an injection of the liposomal drug which then gets released at sites where alpha toxin activity has been reconstituted. The rationale for the two-fragment approach is that alpha-toxin happens to be the lethal toxin secreted by *C. perfringens*. Using a two-fragment approach minimizes the possibility of lethality caused by systemic hemolysis. Data pertinent to this patent has been published in *Int J Oncol. 1998 Oct;13(4):819-25*. No total eradication of tumors was observed in that paper. Liposome-enabled drug-delivery is distinguished from the current patent as it is in a different enzyme class and uses an unrelated non-catalytic mechanism.

5,718,915 Antiviral liposome having coupled target-binding moiety and hydrolytic enzyme

This patent describes a method for targeting antiviral liposomes by coupling lipases (among other hydrolytic enzymes) which would target and digest viral protein components, increasing access of the liposomal drug to the pathogen in question. This is not a method for triggered liposomal release as the coupled lipase must avoid using the liposome as a substrate.

3. Search terms: "Hemolysin" AND "Liposomes"

5,643,599 Intracellular delivery of macromolecules

This patent involves the inclusion of hemolysins within the lumen of the liposome to create a 'ticking time bomb' so that the liposome self-destructs within a time span thus allowing the liposomal drug contents to escape prior to degradation in lysosomes. This release is time-dependent and non-specific.

Literature Review and Search

A brief review of liposome technology (Please skip if familiar)

One early attempt to reduce systemic toxicity employed liposomes to encapsulate cytotoxic drugs¹. However, these liposomes were rapidly eliminated by the Mononuclear Phagocyte System (MPS). This limitation was abrogated by the use of a unique liposomal formulation combining PEGylated phospholipids, high cholesterol content (40-50% mol/mol) and hydrogenated phospholipids²⁻⁶. These liposomes, called Sterically-Stabilized Liposomes (SSLs) had several characteristics. The abundant cholesterol and use of hydrogenated phospholipids resulted in a high bilayer phase-transition temperature and general physical rigidity, thus preserving the physical integrity of these liposomes as they traversed the circulation⁷⁻¹³. Additionally, PEGylation markedly reduced the uptake of these liposomes by the MPS, resulting in an improved circulation half-life^{14,15}. This extended circulation time opened a new possibility - exploiting the fenestrations in tumor vessels resulting from aberrant angiogenesis. These pores, varying between 100 to 780 nm in diameter, are significantly larger than the gaps found in normal endothelium which are typically less than 6 nm wide¹⁶. Liposomes could thus be sized large enough to be excluded from normal tissues, yet small enough to passively infiltrate tumor endothelium and deliver their cargo. This phenomenon, called the Enhanced Permeability and Retention (EPR) effect, combined with the impaired lymphatic drainage in tumors, was found to incrementally increase the exposure of tumor to the liposomal drug¹⁴. Thus, SSLs exhibited low toxicity and consequently, the ability to be administered at higher drug doses. Doxil[®], a clinically used SSL formulation of doxorubicin with a greatly improved cardiotoxicity profile, is the prototype example. More recently, tumor-targeting molecules (e.g. antibodies, integrins, folate) have been conjugated to these liposomes to improve their retention within tumors expressing the cognate markers¹⁷.

Paradoxically, the physical robustness of SSLs, a desirable trait for keeping the drug compartmentalized and sequestered, also proved to be a double-edged sword. The SSLs retained in tumors degraded at a slow rate, leaking their therapeutic cargo over a prolonged period of time, therefore making it difficult to achieve the high concentrations necessary for provoking a therapeutic response⁹. This explains why the inherent low toxicity of the SSL approach did not intuitively translate into dramatically improved efficacy, even though greater treatment doses than before were now possible¹⁸⁻²⁵. Consequently, a recent direction of liposome research has been to engineer liposomes that would be selectively destabilized within tumors.

Literature pertaining to triggered release of liposomes

One approach to effect this destabilization involved designing liposomes which incorporated pH-sensitive phospholipids and ligands that bind internalizing receptors highly expressed on tumor cells. Binding of these targeted liposomes to their cognate receptors initiates internalization of the liposomes by receptor-mediated endocytosis. Subsequently, the pH-sensitive liposomal components become destabilized within the acidified endosomal compartments augmenting the release of encapsulated drugs²⁶. These liposomal formulations (even with the inclusion of PEGylated lipids) have significantly shorter circulation times than SSLs, but this is offset by the potential benefit of more liposomal drug being released and thus made bioavailable. A second example exploits Phospholipase A2 (PLA₂), a lipid-metabolizing enzyme elevated in tumor tissue²⁷. However, PLA₂ is not expressed exclusively in tumor tissue. Another limitation of this approach is that PLA₂ is unable to degrade SSLs because of their high (~50%) cholesterol content. Decreasing this cholesterol level to 20% or less would make SSLs degradable by PLA₂, but would also decrease their rigidity and drug retention

capability. Alternative approaches depend on an external source of destabilization directed towards the tumor. This destabilization may be caused by physical means, for example, ultrasound, light, or hyperthermia directed from external sources¹⁷. The drawback to these approaches is that prior knowledge of tumor location is required, which makes this method unsuitable for treating disseminated disease.

Novelty and non-obviousness

Liposomase-enabled drug delivery is an invention which is distinguishable from the above patents and academic literature at many levels. This is the first instance of the combined use of bacteria and liposomes for the purpose of targeted drug delivery. In this incarnation, we are able to benefit from the physical robustness of sterically-stabilized liposomes with their low toxicity profile, while simultaneously piggybacking off the exquisite specificity of *C. novyi-NT* for tumors.

Further, the identification of liposomase opens the door to therapeutic strategies in addition to those based on bacteria. For example, liposomase could be attached to antibodies or encoded within vectors used for gene therapy. As virtually any therapeutic agent can be packaged in liposomes and can thereby act as a "prodrug", liposomase offers a number of interesting possibilities for the specific delivery of drugs to tumors. Remarkably, while lipases (including liposomase) are generally unable to hydrolyze phospholipids, experimental evidence suggests that liposomase disrupts liposomes using a physical mechanism. This is not an intuitively obvious phenomenon. It is therefore not surprising that the use of neutral lipases for the triggered release of liposomal drugs has never been documented.

References

1. Gregoriadis, G., Wills, E. J., Swain, C. P. & Tavill, A. S. Drug-carrier potential of liposomes in cancer chemotherapy. *Lancet* 1, 1313-6 (1974).
2. Blume, G. & Cevc, G. Liposomes for the sustained drug release in vivo. *Biochim Biophys Acta* 1029, 91-7 (1990).
3. Klibanov, A. L., Maruyama, K., Torchilin, V. P. & Huang, L. Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. *FEBS Lett* 268, 235-7 (1990).
4. Senior, J., Delgado, C., Fisher, D., Tilcock, C. & Gregoriadis, G. Influence of surface hydrophilicity of liposomes on their interaction with plasma protein and clearance from the circulation: studies with poly(ethylene glycol)-coated vesicles. *Biochim Biophys Acta* 1062, 77-82 (1991).
5. Papahadjopoulos, D. et al. Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. *Proc Natl Acad Sci U S A* 88, 11460-4 (1991).
6. Lasic, D. D., Martin, F. J., Gabizon, A., Huang, S. K. & Papahadjopoulos, D. Sterically stabilized liposomes: a hypothesis on the molecular origin of the extended circulation times. *Biochim Biophys Acta* 1070, 187-92 (1991).
7. Allen, T. M. & Cleland, L. G. Serum-induced leakage of liposome contents. *Biochim Biophys Acta* 597, 418-26 (1980).
8. Bally, M. B. et al. Liposomes with entrapped doxorubicin exhibit extended blood residence times. *Biochim Biophys Acta* 1023, 133-9 (1990).
9. Horowitz, A. T., Barenholz, Y. & Gabizon, A. A. In vitro cytotoxicity of liposome-encapsulated doxorubicin: dependence on liposome composition and drug release. *Biochim Biophys Acta* 1109, 203-9 (1992).
10. Hunt, C. A., Rustum, Y. M., Mayhew, E. & Papahadjopoulos, D. Retention of cytosine arabinoside in mouse lung following intravenous administration in liposomes of different size. *Drug Metab Dispos* 7, 124-8 (1979).
11. Lang, J., Vigo-Pelfrey, C. & Martin, F. Liposomes composed of partially hydrogenated egg phosphatidylcholines: fatty acid composition, thermal phase behavior and oxidative stability. *Chem Phys Lipids* 53, 91-101 (1990).
12. Mayhew, E., Rustum, Y. M., Szoka, F. & Papahadjopoulos, D. Role of cholesterol in enhancing the antitumor activity of cytosine arabinoside entrapped in liposomes. *Cancer Treat Rep* 63, 1923-8 (1979).
13. Papahadjopoulos, D., Nir, S. & Oki, S. Permeability properties of phospholipid membranes: effect of cholesterol and temperature. *Biochim Biophys Acta* 266, 561-83 (1972).
14. Yuan, F. et al. Microvascular permeability and interstitial penetration of sterically stabilized (stealth) liposomes in a human tumor xenograft. *Cancer Res* 54, 3352-6 (1994).
15. Gabizon, A. A., Barenholz, Y. & Bialer, M. Prolongation of the circulation time of doxorubicin encapsulated in liposomes containing a polyethylene glycol-derivatized phospholipid: pharmacokinetic studies in rodents and dogs. *Pharm Res* 10, 703-8 (1993).
16. Drummond, D. C., Meyer, O., Hong, K., Kirpotin, D. B. & Papahadjopoulos, D. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. *Pharmacol Rev* 51, 691-743 (1999).
17. Andresen, T. L., Jensen, S. S., Kaasgaard, T. & Jorgensen, K. Triggered activation and release of liposomal prodrugs and drugs in cancer tissue by secretory phospholipase A2. *Curr Drug Deliv* 2, 353-62 (2005).
18. Ellerhorst, J. A. et al. Phase II trial of doxil for patients with metastatic melanoma refractory to frontline therapy. *Oncol Rep* 6, 1097-9 (1999).

19. Garcia, A. A., Kempf, R. A., Rogers, M. & Muggia, F. M. A phase II study of Doxil (liposomal doxorubicin): lack of activity in poor prognosis soft tissue sarcomas. *Ann Oncol* 9, 1131-3 (1998).
20. Halford, S. et al. A phase II study evaluating the tolerability and efficacy of CAELYX (liposomal doxorubicin, Doxil) in the treatment of unresectable pancreatic carcinoma. *Ann Oncol* 12, 1399-402 (2001).
21. Matsumura, Y. et al. Phase I and pharmacokinetic study of MCC-465, a doxorubicin (DXR) encapsulated in PEG immunoliposome, in patients with metastatic stomach cancer. *Ann Oncol* 15, 517-25 (2004).
22. Muggia, F. M., Blessing, J. A., Sorosky, J. & Reid, G. C. Phase II trial of the pegylated liposomal doxorubicin in previously treated metastatic endometrial cancer: a Gynecologic Oncology Group study. *J Clin Oncol* 20, 2360-4 (2002).
23. Harrington, K. J. et al. Phase I-II study of pegylated liposomal cisplatin (SPI-077) in patients with inoperable head and neck cancer. *Ann Oncol* 12, 493-6 (2001).
24. Kim, E. S. et al. A phase II study of STEALTH cisplatin (SPI-77) in patients with advanced non-small cell lung cancer. *Lung Cancer* 34, 427-32 (2001).
25. White, S. C. et al. Phase II study of SPI-77 (sterically stabilised liposomal cisplatin) in advanced non-small-cell lung cancer. *Br J Cancer* 95, 822-8 (2006).
26. Simoes, S., Moreira, J. N., Fonseca, C., Duzgunes, N. & de Lima, M. C. On the formulation of pH-sensitive liposomes with long circulation times. *Adv Drug Deliv Rev* 56, 947-65 (2004).
27. Davidsen, J., Vermehren, C., Frokjaer, S., Mouritsen, O. G. & Jorgensen, K. Drug delivery by phospholipase A(2) degradable liposomes. *Int J Pharm* 214, 67-9 (2001).