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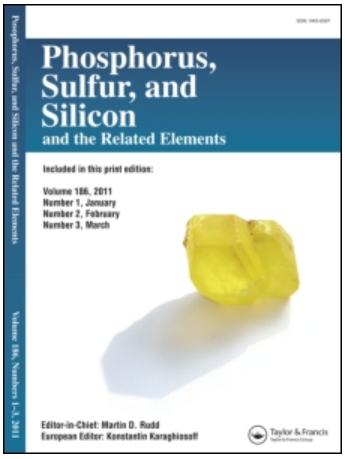

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STERICALLY STABILIZED DOXORUBICIN LOADED LIPOSOMES (DOX-SL™): FROM BASICS TO THE CLINICS

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Abstract

This short paper describes the problems, and their solutions, of formulating liposomal dosage forms for clinical use, as exemplified in tumor therapy by liposomal doxorubicin. We will demonstrate that the development of an efficacious drug delivery system requires fitting the delivery system to the micro-anatomy of the tumor. Many tumors have gaps in their capillaries which permit particles smaller than 120 nm to slowly extravasate into the tumor. Therefore small liposomes having a prolonged circulation time may be suitable drug carriers for tumor therapy. Recently a liposomal doxorubicin formulation (DOX-SL™) was recommended for approval by the US FDA, with indications of treating Kaposi's sarcoma. These liposomes seem to be efficacious in other types of tumors as well.The unique features of this formulation include: control of the biodistribution and pharmacokinetics of the intact liposomes through liposome lipid composition, especially the role of steric stabilization of the liposomes which enable prolonging their circulation time (human $t_{1/2}$ > 50 h); stable loading of a sufficient level of drug through an ammonium sulfate gradient; and small particle size (100 nm) These special features enable the development of liposomal doxorubicin which in humans delivers the active drug to the tumor more efficiently than the use of the free drug. These novel liposomal formulations may provide the means for realizing Paul Erlich's dream of active targeting by a "magic bullet".

Key Words: tumor targeting, sterically stabilized liposomes, doxorubicin, ammonium sulfate gradients, drug loading, extravasation.

INTRODUCTION

The main advantage of a drug carrier is its ability to modify the pharmacokinetics and biodistribution of the drug, so that the drug level at the target is sufficient for therapeutic benefits, and low in the non relevant tissues. This can be described by three parameters, two of them, the drug-to-carrier partition coefficient ($K_{p,c}$) and the rate of drug release from the carrier (k_{off}), are related to drug–carrier interactions; the third one is the rate of carrier clearance (k_c). We demonstrate that carrier performance for drugs associated with the carrier amphiphile(s) is determined to a large extent by $K_{p,c}$, while for drugs encapsulated in the aqueous phase of the carrier it is important that k_{off} will be similar to k_c. These conclusions are based on two examples,of liposomal doxorubicin which consisted of either doxorubicin associated with the membrane of negatively-charged, fluid oligolamellar liposomes (L-DOX), or doxorubicin loaded by an ammonium sulfate gradient into small, unilamellar, rigid liposomes having steric stabilizing lipid grafted in their lipid bilayer, (S-DOX).

RESULTS AND DISCUSSION

I. Doxorubicin in Liposomes: System Characterization

For liposome associated drug the mode of drug association with the liposomes is controlled by the combination of $K_{p,c}$ and k_{off} which have major effect on the stability of in vivo encapsulation. Combining optimization of liposome composition and size determine k_c and the ability to reach extrahepatic tumors and inflammation sites (1,2). We take advantage that the same drug (doxorubicin) was used with 2 different liposomal formulations as described by Amselem et al. (3) and Gabizon et al. (4). For both formulations studies were performed which include in vitro characterization, toxicity, pharmacokinetic and efficacy studies in mice (rev. in 3,5) and in human (3,4,6,7).

The major relevant differences between the 2 formulations is that L-DOX are oligolamellar, negatively-charged, fluid liposomes having the drug (DOX) associated with the liposome bilayers (5,8). After i.v. injection it concentrates in the RES of the mice (8). The S-DOX are smaller-size unilamellar rigid liposomes which contain a steric stabilizing lipid ^{2000}PEG-DSPE grafted in the bilayer (being attached to the amino group of distearoyl phosphatidylethanolamine (DSPE)) which reduces uptake by the RES and therefore prolongs their circulation time (1,2,4,7). The S-DOX doxorubicin is encapsulated in the intraliposomal aqueous phase as a DOX-sulfate precipitate (9,10,11,12). The main differences between these 2 formulations in their performance in humans is related to the large differences in their $K_{p,c}$, k_{off}, and k_c .

II. Comparison Between the Pharmacokinetic Performance of L-DOX and S-DOX in Humans

The detailed pharmacokinetic performance of L-DOX and S-DOX in humans is described elsewhere (6,7). It demonstrates drastic (2–3 orders of magnitude) differences in the plasma level of DOX upon administration of equal doses of 50 mg/m^2 body surface area of the 2 formulations. The DOX delivered as L-DOX is cleared much faster, with a typical biphasic kinetics, large volume of distribution, and distribution $t_{1/2} << 10$ min. (7). The level of plasma DOX measured 5 min after L-DOX infusion is 10-fold lower than the level of drug in plasma 24 h after S-DOX injection. The pharmacokinetics of DOX delivered as L-DOX resembles more the behavior of free drug than liposome-associated drug. Careful examination of L-DOX pharmacokinetics, especially the clearance of liposome-associated drug and the liposomes reveals that after the i.v. injection more than 60% of the drug was released in human plasma in less than 1 s (3,6). This fast release rate can be explained by 3000 fold dilution induced release in vitro. During the i.v. infusion of L-DOX to humans the dilution varied in the range of 1000–200 in the beginning and the end of the infusion respectively, followed by 2000-fold dilution 3 h post infusion. Based on the DOX liposomes/buffer dilution-dependent $K_{p,c}$ of 4500 to 60000 (for low and high dilution respectively, 3) such a high level of release is expected to occur during the infusion. The fast release rate indicates that k_{off} is very short, as was also found for the in vitro release. The $t_{1/2}$ of the L-DOX liposomes (the carrier) is about 30 min, but of the drug is much shorter, namely that k_{off} is shorter than k_c, and k_{off} determines the clearance of the drug delivered as L-DOX. The DOX delivered as S-DOX has a small volume of distribution (7), which is slightly larger than the plasma volume. Its clearance is close to monophasic and is much slower: 168 h post infusion the plasma still contain 6–8% of the injected dose (which is slightly higher than the level achieved 5 min after the injection of an equal dose of L-DOX). For S-DOX, in spite of the low $K_{p,c}$, there is almost no drug release, and the fact that k_{off} is slower than k_c indicates that k_{off} is in the same order of magnitude as k_c. Then the carrier determines the pharmacokinetics of the drug in the human circulation. We found that the release kinetics of DOX induced by 3000-fold dilution of either L-DOX or S-DOX is very different. While the release of

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DOX from L-DOX was almost instantaneous, from S-DOX it was at least million times slower. What determines the low k_{off} obtained for DOX-SL? From studies of us and others it is clear that it is a combination of the use of ammonium sulfate for the drug loading together with the high "rigidity" of the liposome bilayer (composed of HPC:cholesterol:PEG-DSPE) A more careful examination of the role of the ammonium gradient reveals that DOX-sulfate is precipitated in the liposomal aqueous phase as bundles of parallel fibers, Solubility product (K_s) of DOX-sulfate is low to the extent that in S-DOX 98% of the loaded drug is in the intraliposomal precipitate. These physical features explain the much high stability of DOX encapsulation of S-DOX (12), which explain the very long circulation time of S-DOX (DOX-SL).

III. Implications on Liposome Performance

Long cirulation time of liposomes loaded with drug is the first requirement for efficacy. The other requirments to be met include the abiltiy to extravasate to the tumor tissues, and obtaining sufficient level of bioavaiable drug in the tumor cells. This was indeed acheived for DOX-SL as demonstrated by our clinical studies which show that DOX-SL deliver 3-10 times more DOX to the tumor site than free drug (7). This is explained by the extravasation of of the DOX-SL vesicles which are ~100 nm in diameter, a size which enable selective "slipping" through the gaps in the tumor vascular system (7). Using the follow up of the intracellular metabolism of DOX we demonstrate that DOX delivered via DOX-SL is bioavailable to the tumor cells (7). All the above explain the superior terapeutic efficacy of DOX-SL over free DOX which led to the recent recommendation for approval for clinical use with indication to Kaposi's sarcoma. This unique properties of DOX-SL also enable to revisit the Paul Ehrlich concept of the "Magic Bullet" as exemplify by the passive targeting discussed here or by immunotargeting (13).

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Abbreviations: DOX, doxorubicin; PC, phosphatidylcholine; L-DOX, doxorubicin associated with egg PC:egg PG:cholesterol oligolamellar vesicles; S-DOX (DOX-SL™), doxorubicin remote loaded into sterically stabilized small unilamellar vesicles; ; RES, reticuloendothelial system; SSL, sterically stabilized liposomes.

REFERENCES

1. Woodle, M. C. and Lasic, D. D., Sterically stabilized liposomes. Biochim. Biophys. Acta, 1113, 171, (1992).
2. Lasic, D. D. and Martin, F. J., Stealth Liposomes, CRC Press, Boca Raton, FL(1995).
3. Amselem, S., Cohen, R., and Barenholz, Y., In vitro tests to predict in vivo performance of liposomal dosage forms, Chem. Phys. Lipids 64, 219 (1993).
7. Gabizon, A., Isacson, R., Libson, E., Kaufman, B., Uziely, B., Catane, R., Gera Ben Dor, C., Rabello, E., Lass, Y., Peretz, T., Sulkes, A., Chisin, R., and Barenholz, Y., Acta Oncol. 33, 779, (1994).
5. Barenholz, Y., Amselem, S., Goren, D., Cohen, R., Gelvan, D., Samuni, A., Golden, E. B., and Gabizon, Medicinal Res. Rev. 13, 449, (1993).
6. Gabizon, A., Chisin, R., Amselem, S., Druckmann, S., Cohen, R., Goren, D., Fromer, I., Peretz, T., Sulkes, A., and Barenholz, Y., Br. J. Cancer, 64, 1125, (1991).
4 Gabizon, A., Catane, R., Uziely, B., Kaufman, B., Safra, T., Cohen, R., Martin, F., Huang, A., and Barenholz, Y., Cancer Res. 54, 987, (1994).,
8. Goren, D., Gabizon A., and Barenholz, Y., Biochim. Biophys. Acta 1029, 285, (1990).

9. Haran, G., Cohen, R., Bar, L. K., and Barenholz, Y., Biochim. Biophys. Acta 1151, 201 (1993).
10. Lasic, D. D., Frederik, P. M., Stuart, M. C. A., Barenholz, Y., and McIntosh, T. J., FEBS Lett., 312, 255 (1992).
11 Bolotin, E. M., Cohen, R., Bar, L. K., Emanuel, N., Ninio, S., Lasic, D. D., and Barenholz, Y., J. Liposome Res. 4, 455 (1994).
12. Lasic D., Ceh, B., Stuart, M. C., Guo, L., Frederik, P. M., and Barenholz Y., Biochim. Biophys Acta, in press (1995.)
13. Emanuel, N., Kedar, E., Bolotin, E. M., Smordinsky, N. I., and Barenholz, Y., Pharm. Res., accepted for publication (1995).

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