DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 14(1), 143-153 (1988)

THE LEAKAGE CHARACTERISTICS OF LIPOSOME-ENCAPSULATED ADRIAMYCIN-DEXTRAN CONJUGATES

S.L. LAW*, W.Y. LO** and G.W. TEH

* Pharmaceutics Research Laboratory, Department of Medical Research, Veterans General Hospital, Taipei, Taiwan, R. O. C.

** Copley Pharmaceutical Inc., Boston, Ma 02127, U. S. A.

ABSTRACT

The leakage characteristics of liposome-encapsulated adriamycin-dextran conjugates was investigated. The results of in vitro studies indicate that the leakage of adriamycin-dextran T10 conjugate and adriamycin from liposomes was a diffusion process. The results of in vivo studies show that the half-life of plasma clearance of adriamycin-dextran conjugates was greatly enhanced. The larger molecular size of the encapsulated drugs caused a slower half-life of clearance.

This probably resulted from a slower rate of diffusion.

INTRODUCTION

One of the problems in the development of liposomes as carriers for drugs is the leakage of encapsulated drugs to the enviroment. In vitro leakage causes inaccuracy of dosage. In vivo leakage leads to loss of carrier effect of liposomes and induction of side effect of drugs. There are several methods used to control leakage of drugs from liposomes, such as modification of membrane structure by using different lipid components or compositions[1,2], interaction of drugs with membrane components by lipophilic interaction or charge interaction etc [3,4], and formation of large molecular complexes by associating drugs with polymers [5,6].

The binding of drugs to polymers has the effect of targeting delivery [7]. As a drug-polymer is compounded with liposomes, it is expected to increase the effect of targeting delivery and prevent leakage of drugs from liposomes.

In the encapsulation of drug-polymers in liposomes, it is suggested that the molecular size of the encapsulated materials should be one of the

factors affecting leakage. However, little work has been published describing this factor.

Adriamycin is a broad spectrum anticancer agent[8]. It is frequently encapsulated in liposomes for the development of cancer chemotherapy [9,10,11,12]. Therefore, in this first communication the in vitro and in vivo leakage characteristics of liposome-encapsulated adriamycin-dextran conjugates are reported. The leakage characteristics were related to the molecular size of the encapsulated drugs.

MATERIALS AND METHODS

Adriamycin (Farmatalia, Carlo Erba, Italy), sodium borohydride (Merck, Germany), sodium periodate (Merck, Germany), Sephadex G-100 (Pharmacia, Sweden), dicetyl phosphate (P.L. Biochemical Inc., U.S.A.), cholesterol (Sigma, U.S.A.), dextran T10 (M.W. 10,000), dextran T40 (M.W. 40,000) and dextran T70 (M.W. 70,000) (Pharmacia, Sweden) were used as received. Phosphatidyl choline was prepared according the methods of Hanahan et al [13] and Singleton et al [14] as described previously [15].

Preparation of adriamycin-dextran conjugates

The conjugated drugs were prepared according to the methods of Bernstein et al [16]. In brief, dextran interacted with sodium periodate to 50% oxidation.

Adriamycin was added to form Schiff base with the oxidized dextran in a phosphate buffer saline (pH 7.2) at the weight ratio of 1:5 of drug to dextran in the dark overnight and then reduced by sodium borohydride. The adriamycin-dextran conjugates were eluted through Sephadex G-100 to separate from the unbound adriamycin. The eluent of the conjugates was checked at 475 nm to obtain a concentration using adriamycin as standard.

Encapsulation of drugs in liposomes

Phosphatidyl choline cholesterol and dicetyl phosphate were dissolved in chloroform at the molar ratio of 1.6:1:0.15 and dried under reduced pressure at 37°C to form a thin film in a round bottom flask. Drugs dissolved in phosphate buffer saline were added to the film and vortexed for five minutes. Multilamellar liposomes encapsulated with drugs were obtained. The multilamellar liposomes were washed with phosphate buffer saline three times by centrifuging at 32,000g for half hour to separate them from the free drugs and small liposomes.

In vitro leakage of drugs from liposomes

In vitro leakage of drugs from liposomes was carried out after allowing the liposomes to stand for a certain period of time at a constant temperature of

25° C. At various time intervals, the liposomes were centrifuged at 127,000g for one hour to obtain a clear supernatant. The supernatnat was checked for adriamycin concentration by fluorimetric method with an excitation maximum at 470 nm and an emission maximum at 550 nm. Duplicate samples were made for each determination.

Plasma clearance of free and encapsulated drugs

Female Sprague-Dawley rats weighing 250-350g were used. The rats were anesthetized with thiopentone sodium and injected in the jugular vein with free and encapsulated drugs at a dose of 200 µg adriamycin per kg. At various time intervals, blood samples were withdrawn from the carotid artery and the rats were sacrificed. Three to five rats were used for each determination. To determine the blood concentration of adriamycin, the method of Chan and Harris was used [17]. Fluorimetric measurements were made at 470 and 550 nm for excitation and emission maximum respectively.

RESULTS AND DISCUSSION

Liposomes which consisted of phosphatidyl choline, cholesterol and dicetyl phosphate at the molar ratio of 1.6:1:0.15 were constructed according to the composition of red blood cells i.e. the cholesterol comprises about 23% w/w of total

membrane lipid [18] and the negative surface charge content is equivalent to an electrophoretic mobility of 1.31 μ/sec per v/cm [15] . Experiments indicated that with this composition retention of drugs in liposomes showed a greatest stability [21] . It is suggested that liposomes with composition like red blood cells are suitable for a drug delivery system.

The results of in vitro leakage for adriamycin and adriamycin-dextran T10 conjugate from liposomes are shown in Figure 1 which is expressed as the amount of drug leakage against the square root of time. The adriamycin-dextran T10 conjugate leakage from liposomes shows a good straight line. This indicates that the drug leakage from liposomes is mainly a diffusion mechanism [19] . For adriamycin leakage from liposomes, there are two straight lines of a biphasic system. The drugs exhibit a rapid diffusion in the first phase and follow a second phase of slow diffusion. In the first phase, the rapid diffusion process is probably due to the release of the adsorbed or intercalated drugs on or in the outer bilayers of the liposomes [6,20] . The slow phase of diffusion may be due to the leakage of the drugs from the inner aqueous phase of the liposomes. In the case of

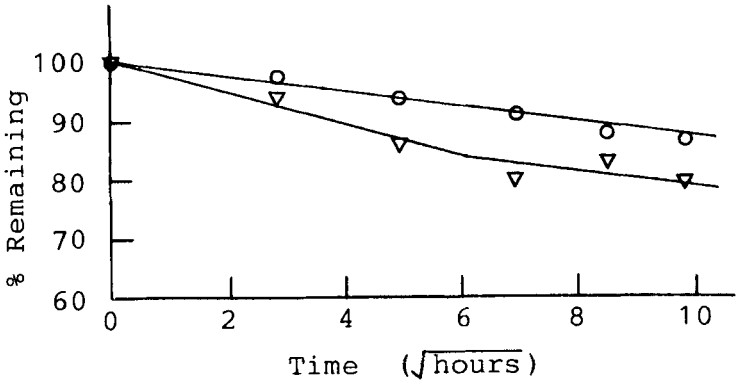

FIGURE 1

In vitro leakage of free adriamycin (∇) and adriamycin-dextran T10 conjugate (o) from liposomes.

adriamycin-dextran T10 conjugate in liposomes, the drugs probably transport directly from the inner aqueous phase through the bilayer membrane to the enviroment.

The plasma concentration against time plots for the free adriamycin, and adriamycin and adriamycin-dextran conjugates encapsulated in liposomes are shown in Figure 2. All curves show a biphasic clearance profile i.e. there is a rapid phase of initial drug clearance and a slow phase of gradual elimination. The values for the half-life of clearance from the rapid phases are 0.5 hour for free adriamycin, and 1.1, 2.6, 3.3 and 23.1 hours for adriamycin , adriamycin-dextran T10 conjugate, adriamycin-dextran T40 conjugate and adriamycin-dextran T70 conjugate in

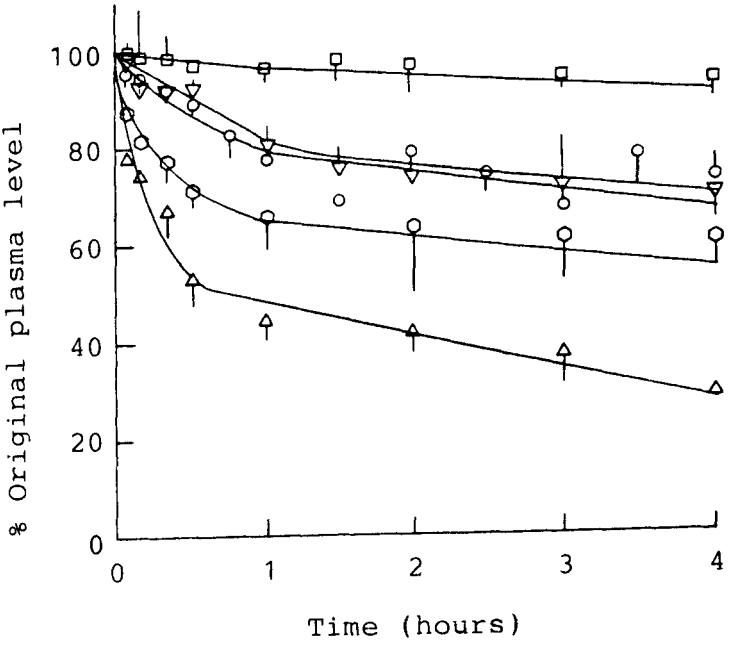

FIGURE 2

Plasma concentrations of free adriamycin (△), and adriamycin (○), adriamycin-dextran T10 conjugate (○), adriamycin-dextran T40 conjugate (▽) and adriamycin-dextran T70 conjugate (□) in liposomes.

liposomes respectively. The values for the half-life of clearance from the slow phases are 4.3 hours for free adriamycin, and 11.6, 17.3, 23.1 and 69.3 hours for adriamycin, adriamycin-dextran T10 conjugate, adriamycin-dextran T40 conjugate and adriamycin-dextran T70 conjugate in liposomes respectively. The calculation of the values of half-life was made according to the first order reaction equation. It shows that drugs encapsulated in liposomes have a longer half-life of clearance than the free drug. This

is in agreement with the results of liposome encapsulated anticancer drugs[5,9,22]. When dextrans were conjugated to adriamycin, the half-life of clearance was greatly enhanced. It seems that a larger molecular size of the encapsulated drugs results in a slower half-life of clearance, although the difference between the data for adriamycin-dextran T10 conjugate and adriamycin-dextran T40 conjugate is not significant. The dependence of molecular size of the encapsulated drugs on the clearance may be due to the larger the molecular size, the slower the rate of diffusion through the liposome membrane.

The present findings suggest that adriamycin conjugated with dextrans prevents leakage from liposomes and its effect is more significant with the higher molecular weight of dextrans. Further study is necessary in order to understand the drug distribution in tissues.

ACKNOWLEDGMENT

This work was supported by the National Science Council R.O.C. (NSC 74-0412-B075-39).

REFERENCES

1. J. Senior and G. Gregoriadis, in "Liposome Technology , Vol. III, Targeted Drug Delivery

and Biological Interaction," G. Gregoriadis, eds., CRC Press Inc, Florida, 1984, p.263.

2. S.L. Regen, A. Singh, G. Oehme and M. Singh, Biochem. Biophys. Res. Commun., 101, 131 (1981).
3. C.G. Knight, in "Liposomes: From Physcial Structure to Therapeutic Applications," C.G. Knight, eds., Elsevier, Amsterdam, 1981, p.381.
4. J.H. Fendler, in "Liposomes in Biological Systems," G. Gregoriadis and A.C. Allison, eds., John Wiley and Sons, N.Y., 1980, p.87.
5. G. Gregoriadis, P.J. Davisson and S. Scott, Biochem. Soc. Trans., 5, 1323 (1977).
6. A. Wanichsiriroj, Y.M. Joshi and D.O. Kildsig, Drug Dev. Ind. Pharm., 10, 613 (1984).
7. E.P. Goldberg, "Targeted Drugs," John Wiley and Sons Inc., N.Y., 1983.
8. R.C. Young, R.F. Ozols and C.E. Meyers, N. Engl. J. Med., 305, 139 (1981).
9. F. Olson, E. Mayhew, D. Maslow, Y. Rustum and F. Szoka, Eur. J. Cancer Clin. Oncol., 18, 167 (1982).
10. R.M. Abra, C.A. Hunt, K.K. Fu and J.H. Peters, Cancer Chemother. Pharmacol., 11, 98 (1983).
11. E. Mayhew, R. Lazo and W.J. Vail, in "Liposome Technology, Vol. II, Incorporation of Drugs,

Proteins, and Genetic Material," G. Gregoriadis, eds., CRC Press Inc., Florida, 1984, p.19.

12. A. Rahman, G. White, N. More and P.S. Schein, Cancer Res., 45, 796 (1985).

13. D.J. Hanahan, M.B. Turner and M.E. Jayko, J. Biol. Chem., 192, 623 (1951).

14. W.S. Singleton, M.S. Gray, M.L. Brown and J.L. White, J. Am. Oil Chem. Soc., 42, 53 (1965).

15. S.L. Law, W.Y. Lo, S.H. Pai, G.W. Teh and F.Y. Kou, Intern. J. Pharm., in press.

16. A. Bernstein, E. Hurwitz, R. Maron, R. Arnon, M. Sela and M. Wilchek, J. Natl. Cancer Inst., 60, 379 (1978).

17. K.K. Chan and P.A. Harris, Res. Comm. Chem. Path. Pharm., 6, 447 (1973).

18. B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts and J.D. Watson, "Molecular Biology of the Cell," Garland Publishing Inc., N.Y., 1983, p.266.

19. T. Higuchi, J. Pharm. Sci., 50, 874 (1961).

20. D.J.A. Crommelin, N. Slaats and L. van Bloois, Intern. J. Pharm., 16, 79 (1983).

21. S.L. Law, W.Y. Lo and G.W. Teh, Unpublished results.

22. R.L. Juliano and D. Stamp, Biochem. Pharm. 27, 21 (1977).