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Release characteristics of mitoxantrone-containing liposomes

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Summary

The release characteristics of mitoxantrone-containing liposomes was studied. The effect of lipid composition on the release rate showed an order of positively charged liposomes > neutral liposomes > negatively charged liposomes. This was attributed to the strength of the binding forces involved in the interaction between the drug and lipids. The release of mitoxantrone from liposomes after the incorporation of galactocerebroside demonstrated no dependence on the molar ratio of added galactocerebroside. This was due to the binding force being insufficient to influence the release rate of the entrapped mitoxantrone significantly. The effect of temperature on the release rate revealed that 4°C may be a suitable temperature for storage of the liposomes during the time range studied, since the release rate was slow. At 4, 25 and 37°C, the release profiles fitted a linear concentration-square root of time plot which demonstrated the diffusion-controlled release of entrapped mitoxantrone from the membrane matrix. The effect of pH on release showed an increase in release rate with increasing pH. At high pH, partitioning of mitoxantrone into the membrane matrix was considerable and drug was released rapidly.

Introduction

A previous paper (Law et al., 1991) has dealt with the formulation and loading efficiency of mitoxantrone on liposomes. The results demonstrated that negatively charged liposomes produce a high loading efficiency depending on the ionic strength and pH of the medium. Neutral liposomes showed a profile of loading efficiency similar to that of negatively charged liposomes but with a lower magnitude of loading efficiency. With positively charged liposomes, the loading efficiency increased with the added concentration of mitoxantrone. The effect of galactocerebroside on the loading efficiency increased with added concentration of mitoxantrone. The loading efficiency was dependent on neither the phospholipid concentration of the liposomal membrane, nor the methods for preparing and separating the liposomes.

The present work was undertaken in order to investigate the release characteristics of mitoxantrone-containing liposomes. The effect of phospholipid composition on liposomes, molar concentration of galactocerebroside in the liposomal

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membrane, storage temperature, and pH of the medium on the release of mitoxantrone is reported.

Materials and Methods

Materials

Mitoxantrone was obtained from Kingdom Pharmaceutical Co. (R.O.C.). Phosphatidylcholine (from fresh egg yolk, type XI E), dioleoylphosphatidylcholine, galactocerebroside (from bovine brain, type II) and cholesterol were purchased from Sigma (U.S.A.). Stearylamine and dicetyl phosphate were obtained from Pharmacia P-L Chemicals (Sweden). General chemicals were of analytical grade.

Methods

Preparation of mitoxantrone-containing liposomes

Liposomes were prepared according to the methods described previously (Law et al., 1991). Briefly, the phospholipids were dissolved in chloroform in a 50 ml round-bottom flask and dried in a rotary evaporator under reduced pressure at 37°C to form a thin film on the flask. The desired concentration of mitoxantrone in phosphate-buffered saline of various pH values at an ionic strength of 0.154 was added to the film. Multilamellar liposomes were formed by constant vortexing for 5 min on a vortex mixer (Thermolyne, Syborn, U.S.A.) and sonication for 2 min at 20-s intervals with a probe-type sonicator (Heat Systems Ultrasonics Inc., Model W-220) under an atmosphere of nitrogen. The purpose of sonication of the liposomes was to reduce the particle size rather than to sonicate the multilamellar liposomes to single unilamellar liposomes. The particle size of the liposomes after sonication was reduced from 14 to 5 μm (LPA-3000, Photal, Otsuka Electronics, Japan).

Mitoxantrone-containing liposomes were separated from unentrapped mitoxantrone by ultracentrifugation at $2.8 \times 10^5 \times g$ for 20 min (Beckman TL-100, U.S.A.) and washed three times with buffer. The concentration of entrapped mitoxantrone was determined by lysis of the liposomes with absolute alcohol to obtain a clear solution, followed by measurement at 242 nm.

Release study

Mitoxantrone-containing liposomes were incubated at a temperature of 37°C in several sealed test tubes for the release study. The pH for the release medium was 5.7 and the ionic strength was 0.154. For examination of the effect of temperature on mitoxantrone release, tests were conducted at 4, 25 and 37°C. In the case of the effect of pH on release, pH values of 2.9, 5.7 and 7.0 were studied. Release samples from the test tubes were taken at different time intervals and centrifuged at $2.8 \times 10^5 \times g$ for 20 min to obtain a clear supernatant for measurement of mitoxantrone concentration at 242 nm.

Results and Discussion

The effect of lipid composition on the release of mitoxantrone from liposomes is shown in Fig. 1. Negatively charged liposomes consisting of dioleoylphosphatidylcholine, cholesterol and dicetyl phosphate demonstrated a release profile similar to that of liposomes with a composition of phosphatidylcholine, cholesterol and dicetyl phosphate. It is likely that the change in the phospholipid from dioleoylphosphatidylcholine to phosphatidylcholine does not alter the membrane structure of the liposomes, and the interaction of mitoxantrone with lipid molecules. Therefore, this resulted in no difference in the rate of drug release from the liposomes. This result is consistent with the data on loading where the alteration of the phospholipid composition from dioleoylphosphatidylcholine to phosphatidylcholine demonstrated no influence on the loading efficiency of the liposomes (Law et al., 1991). Positively charged liposomes consisting of dioleoylphosphatidylcholine, cholesterol and stearylamine showed a rapid release rate. In contrast, neutral liposomes with dioleoylphosphatidylcholine demonstrated a release rate intermediate between those of negatively and positively charged liposomes. Negatively charged liposomes gave rise to a slow release rate. It is interesting that the results on loading efficiency showed an order of

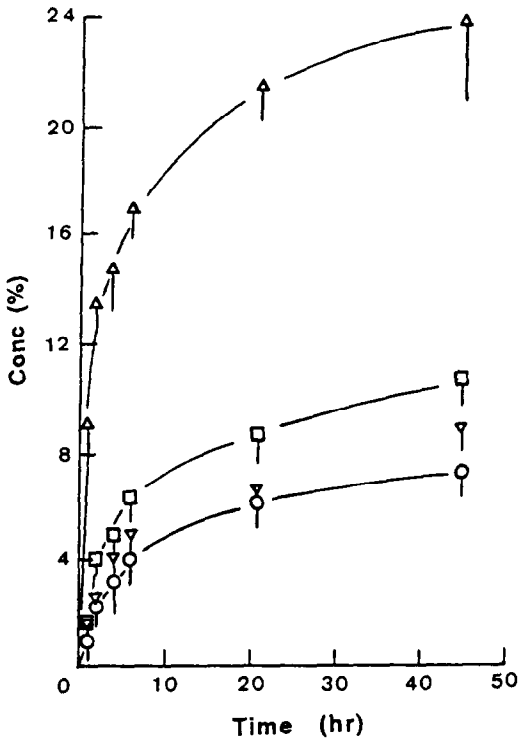

Fig. 1. Effect of lipid composition on release of mitoxantrone from liposomes. (□) Neutral liposomes (DOPC); (○) negatively charged liposomes (DOPC/C/DP 1.6:1:0.15); (△) positively charged liposomes (DOPC/C/S 1.6:1:0.15); (▽) negatively charged liposomes (PC/C/DP 1.6:1:0.15). DOPC, dioleoylphosphatidylcholine; C, cholesterol; DP, dicetyl phosphate; S, stearylamine; PC, phosphatidylcholine.

negatively charged liposomes > neutral liposomes > positively charged liposomes (Law et al., 1991), which is the reverse of the data on the release rate obtained here. That is to say, positively charged liposomes have a fast release rate but a low loading efficiency, and negatively charged liposomes have a slow release rate but a high loading efficiency, neutral liposomes having a medium release rate and loading efficiency. The release and loading characteristics of mitoxantrone-containing liposomes may be due to the strength of the binding forces involved in the interaction of the drug with phospholipids. For example, for positively charged liposomes, an electrostatic attraction between the ionized positively charged mitoxantrone molecules and lipids would not occur. However, for negatively charged liposomes, a strong electrostatic attractive force may exist for the interaction between drug and lipid molecules. Therefore, mitoxantrone in positively charged liposomes has a faster release rate and lower loading efficiency as compared with negatively charged liposomes.

Fig. 2 shows the release profiles of mitoxantrone from negatively charged liposomes after the incorporation of galactocerebroside. Although the addition of various molar ratios of galactocerebroside in the liposomal membrane was carried out, entrapped mitoxantrone demonstrated no significant difference in release rate. As mentioned previously (Law et al., 1991), in the mitoxantrone concentration range below 15 μmol/ml, the loading efficiency was similar at different molar ratios of added galactocerebroside in the liposomes. This is the concentration range of mitoxantrone that was employed here for the preparation of liposomes. Therefore, it is not surprising that the release profiles were similar, while the molar ratio of galactocerebroside incorporated in the liposomes was different. The interaction between mitoxantrone and galactocerebroside molecules may depend on hydrogen bonding (Law et al., 1991). However, at low concentrations of mitoxantrone, although the molar ratio of galactocerebroside in the liposomes is increased, the binding force is insufficient to in-

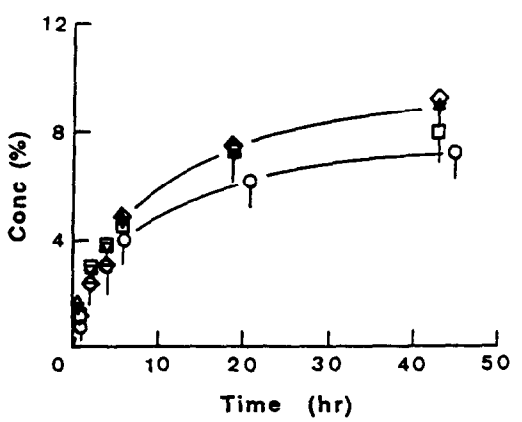

Fig. 2. Effect of incorporation of galactocerebroside into liposomal membranes on release of mitoxantrone from negatively charged liposomes (DOPC/C/DP 1.6:1:0.15). (○) 0.0, (△) 0.2, (□) 0.4, (▽) 0.6, (◇); 1.0 molar ratio of galactocerebroside.

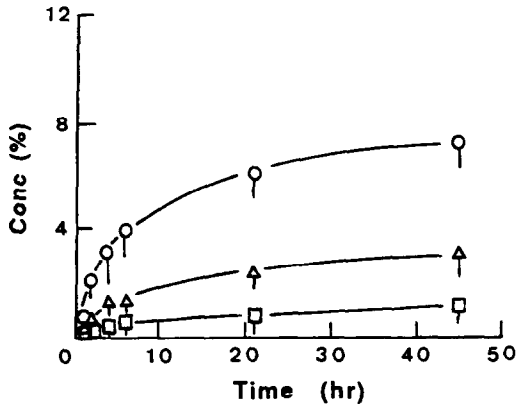

Fig. 3. Effect of temperature on release of mitoxantrone from negatively charged liposomes (DOPC/C/DP 1.6:1:0.15). (□) 4°C, (△) 25°C, (○) 37°C.

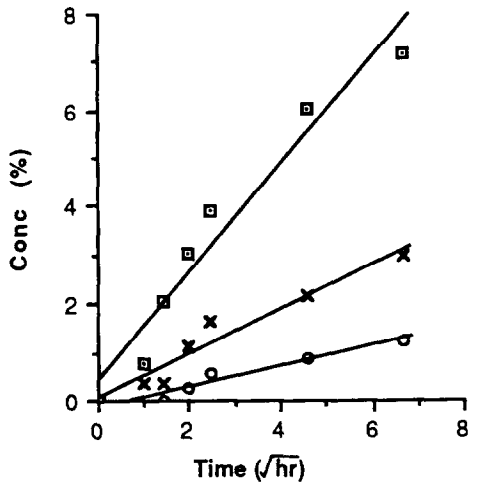

Fig. 4. Release profiles plotted as the percentage of mitoxantrone released vs the square root of time. (○) 4°C, (×) 25°C, (□) 37°C.

fluence the loading capacity and release of loaded mitoxantrone significantly. This may therefore lead to similar results for loading efficiency and release rate.

Fig. 3 presents the release profiles of mitoxantrone from negatively charged liposomes as a function of temperature at 4, 25 and 37°C. As expected, the release rate increased with increasing temperature. It is likely that 4°C is the suitable temperature for the storage of mitoxantrone-containing liposomes, since the release rate was slow and only 1.2% of the total entrapped content was released after storage for 45 h. To examine the mechanism of release of mitoxantrone from liposomes, the percentage of release was plotted vs the square root of time as shown in Fig. 4. It is clear that at 4, 25 and 37°C, the release profiles demonstrate linear behaviour with correlation coefficient of 0.968, 0.962 and 0.964, respectively. This indicates the release of entrapped mitoxantrone from the membrane matrix of the liposomes to be diffusion-controlled process (Higuchi, 1961).

The release profiles of mitoxantrone from negatively charged liposomes at pH values of 2.9, 5.7 and 7.0 are given in Fig. 5. The results showed that the release of mitoxantrone increased with increasing pH. At pH 7.0, there was a dramatic increase in the release rate which was about 3-fold greater than that at pH 5.7. Table 1 lists

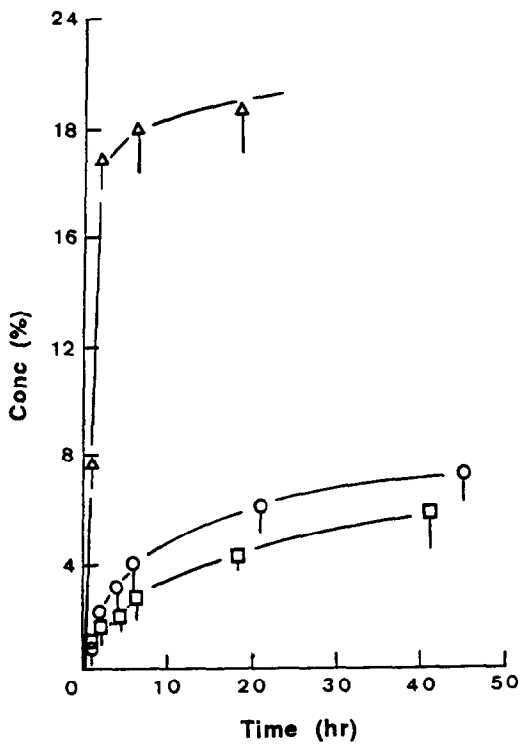

Fig. 5. Effect of pH of the medium on release of mitoxantrone from negatively charged liposomes (DOPC/C/DP 1.6:1:0.15). (□) pH 2.9, (○) pH 5.7, (△) pH 7.0.

TABLE 1

The partition coefficients of mitoxantrone at various pH values

pH	Chloroform/buffer	Octanol/buffer
7.0	1.611	1.477
5.7	0.347	0.404
2.9	0.089	0.094

the results on the partition coefficient of mitoxantrone in the *n*-octanol or chloroform/buffer system at pH 2.9, 5.7 and 7.0. The magnitude of the partition coefficient increased with increasing pH of the buffer system. The pK_a value of mitoxantrone is 7.2. Hence, at pH 7.0, most of the mitoxantrone molecules were unionized and entrapped in the membrane matrix of the liposomes. Partitioning of mitoxantrone into the membrane matrix was considerable at high pH and decreased as the pH decreased. It can be seen from the above results that entrapped mitoxantrone was released slowly at low values of the partition coefficient. The present findings are consistent with the observations of Tsukada et al. (1984), who studied release of the antitumor drugs carboquone, nimustine, 5-fluorouracil and uracil from liposomes, and showed that a low partition coefficient of the drug resulted in a slow release rate. This was attributed to the difficulty of partitioning of the ionized drug into the membrane matrix of the liposomes.

Acknowledgements

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