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Stability of mitoxantrone-containing liposomes

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Abstract

The chemical stability of mitoxantrone entrapped in liposomes was investigated. The effect of silanization on glass container, charge characteristics of the liposomes, pH of the medium, addition of ascorbic acid in the medium, and temperature for storage on the degradation of mitoxantrone followed a pseudo-first-order reaction. The loss of concentration for mitoxantrone was found at zero time of storage in the plain glass vials at pH 5.8 and 7.4. This was attributed to the adsorption of mitoxantrone on the glass vials. At pH 3.6 and 4°C, the mitoxantrone entrapped in negatively charged liposomes in Aquasil-treated glass vials resulted in an optimum half-life. The logarithm of rate constant against pH profiles for mitoxantrone in solution and in liposomes indicated that the degradation rate increased with increasing pH of the medium. This was attributed to a hydroxyl ion catalysis reaction. The results of degradation rate constant obtained from the mitoxantrone entrapped in negatively charged, positively charged and neutral liposomes showed no significant difference. This revealed that a similar stability can result from these systems. The silanization materials of Aquasil and Surfasil on the degradation of mitoxantrone in liposomes resulted in the similar rate constants and half-lives. The addition of ascorbic acid to the mitoxantrone containing liposomes exhibited no effect on the increase of stability for the entrapped mitoxantrone.

Keywords: Mitoxantrone; Dioleoylphosphatidylcholine; Liposome; Chemical stability; Charge characteristics; pH; Temperature; Silanization

1. Introduction

In previous works, the loading efficiency and release characteristics of mitoxantrone-containing liposomes were studied (Law et al., 1991, 1994). It was found that negatively charged liposomes demonstrated 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 liposomal membrane nor the methods for preparing and separat-

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ing the liposomes (Law et al., 1991). The release characteristics of mitoxantrone-containing liposomes showed that the effect of lipid composition on release rate resulted in an order of positively charged liposome > neutral liposome > negatively charged liposome. The release of mitoxantrone from liposomes after the incorporation of galactocerebroside was not dependent on the concentration of added galactocerebroside. The increase in temperature brought about an increase of release rate. At 4, 25 and 37°C, the release profiles fitted a linear concentration-square root of time plot which indicated the diffusion-controlled release of entrapped mitoxantrone from the membrane matrix. At high pH, the partitioning of mitoxantrone into the membrane matrix was considerable and the mitoxantrone was released rapidly (Law et al., 1994).

In the present study, an attempt was made to investigate the chemical stability of mitoxantrone-containing liposomes. The effects of charge characteristics of liposomes, pH of the medium, addition of ascorbic acid as an antioxidant in the medium, silanization materials on the glass vials for storage, and temperature for storage on the degradation of the entrapped mitoxantrone are reported.

2. Materials and methods

2.1. Materials

Mitoxantrone was obtained from Kingdom Pharmaceutical Co. (ROC). Dioleoylphosphatidylcholine and cholesterol were purchased from Sigma (USA). Stearylamine and dicetyl phosphate were obtained from Pharmacia P-L Chemicals (Sweden). Aquasil and Surfasil were purchased from Pierce Co. (USA). General chemicals were of analytical grade and all solvents were of HPLC grade.

2.2. Methods

2.2.1. Preparation of mitoxantrone-containing liposomes

Multilamellar liposomes were prepared according to a method described previously (Law et al., 1991). 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 buffer was added to the film. Multilamellar liposomes were formed by constant vortexing for 5 min on a vortex mixer (Thermolyne, Syborn, USA) and sonication for 2 min at 20-s intervals with a probe-type sonicator (Heat Systems Ultrasonics Inc., Model W-220, USA) 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. Dioleoylphosphatidylcholine was the main lipid component for the construction of liposomes and a molar ratio of 1.6 was used. Cholesterol was added in a molar ratio of 1.0. Liposomes with a positive or negative charge were composed of a 0.15 molar ratio of stearylamine or dicetyl phosphate, respectively.

Mitoxantrone-containing liposomes were separated from the unentrapped mitoxantrone by ultracentrifugation at $2.8 \times 10^5 \times g$ for 20 min (Beckman TL-100, USA) and washed three times with buffer. The concentration of entrapped mitoxantrone was determined by HPLC.

2.2.2. Stability measurement

The buffers used for the stability measurement were pH 3.7 acetate buffer (0.1 M acetic acid and sodium acetate), pH 5.8 phosphate buffer (0.5 M monopotassium phosphate and disodium phosphate), pH 7.4 phosphate buffer (0.5 M monopotassium phosphate and disodium phosphate) and pH 9.6 carbonate buffer (1.0 M sodium hydrogen carbonate and sodium carbonate). The ionic strength of the buffers was adjusted by sodium chloride to a value equivalent to that of 0.9% sodium chloride. In the case of addition of ascorbic acid as antioxidant, the concentration used was 0.5% in the buffers.

Mitoxantrone was found to bind strongly to glassware, and silanization of the glass surface was performed in order to prevent drug adsorption (Ostroy and Gams, 1980; Van Belle et al., 1986; Lin et al., 1989; Hu et al., 1990). Therefore, silanized glassware with Aquasil or Surfasil was

used. Plain glassware was also used in order to study the effect of drug adsorption on glass surface on the degradation of mitoxantrone.

The mitoxantrone-containing liposomes were dispensed and sealed into the Aquasil-treated, Surfasil-treated or untreated glass vials. The samples were stored at 4, 25 and 37°C, and removed at designated times for mitoxantrone concentration analysis.

The stability test of the mitoxantrone solution was also carried out as a reference for the degradation of entrapped mitoxantrone in liposomes. The mitoxantrone solution was diluted with pH 3.6, 5.8, 7.4 and 9.6 buffers and dispensed into the untreated glass vials. The samples were sealed and stored at the temperature of 4, 25 and 37°C. At various time intervals the samples were removed for mitoxantrone concentration analysis.

2.2.3. High-performance liquid chromatography (HPLC) and sample extraction procedure

Mitoxantrone concentrations were determined by HPLC as reported previously (Law and Jang, 1994). A solid-phase extraction procedure using an Aspec system (Gilson, France) and reversed-phase cartridges (Supelclean LC-18 3 ml cartridge, Supelco, USA) was employed for cleaning up the samples.

The calibration curves were constructed by the mean peak-height ratios of the mitoxantrone and the internal standard against the mitoxantrone concentrations in the range from 12.5 to 200 ng/ml. The results were analyzed by linear least-squares regression and almost all the curves showed a correlation coefficient of 0.999.

As described previously (Law and Jang, 1994), the HPLC method was stability-indicating. Also, using this method to detect the entrapped mitoxantrone concentration in liposomes, no interference was found due to the presence of the phospholipids.

3. Results and discussion

Fig. 1 shows the logarithm of concentration of residual mitoxantrone plotted against time for the mitoxantrone solution at pH 3.6, 5.8, 7.4 and 9.6

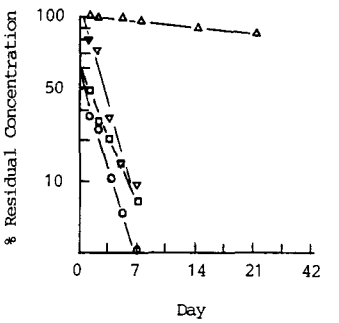

Fig. 1. Concentration-time plots of mitoxantrone in solution at pH 3.6 (△), 5.8 (□), 7.4 (○) and 9.6 (▽) stored in plain glass vials at 25°C.

stored in plain glass vials at 25°C, as representative of the series of temperatures of 4 and 37°C. It is evident that the degradation of mitoxantrone follows pseudo-first-order kinetics. At pH 3.6 and 9.6, the degradation straight lines extrapolated through the residual concentration of mitoxantrone of 100% at zero time of storage. However, for the straight lines at pH 5.8 and 7.4, it showed a 40 and 45% loss of concentration respectively, when extrapolated to time zero. This is possibly due to the adsorption of mitoxantrone on the surface of the plain glass vial. The results of degradation of mitoxantrone obtained at 4 and 37°C, with the effect of pH 5.8 and 7.4 showed a similar profile, however, with lesser and greater adsorption than that at 25°C, respectively. In contrast, at pH 3.6 and 9.6, the extent of adsorption of mitoxantrone on the glass surface was slight.

The degradation of mitoxantrone in solution at pH 3.6, 5.8, 7.4 and 9.6 stored in the Aquasil-treated glass vials at temperatures of 4, 25 and 37°C indicated a pseudo-first-order process (Fig. 2; only the results obtained at 25°C shown). The straight line showed an intercept at a mitoxantrone concentration of 100% at zero time of storage. There was no apparent adsorption of mitoxantrone on the surface of the glass vial.

Tables 1 and 2 list the observed rate constants for the degradation of mitoxantrone at pH 3.6, 5.8, 7.4 and 9.6 stored at temperatures of 4, 25 and 37°C in plain and Aquasil-treated glass vials, respectively. The rate constants were dependent

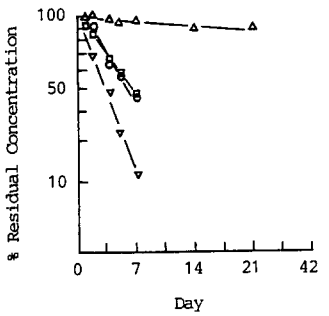

Fig. 2. Concentration-time plots of mitoxantrone in solution at pH 3.6 (△), 5.8 (□), 7.4 (○) and 9.6 (▽) stored in Aquasil-treated glass vials at 25°C.

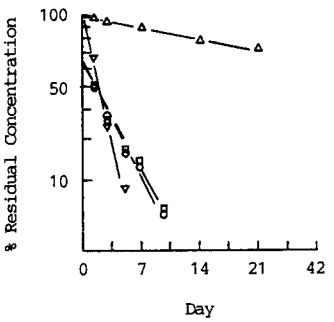

Fig. 3. Concentration-time plots of mitoxantrone entrapped in negatively charged liposomes at pH 3.6 (△), 5.8 (□), 7.4 (○) and 9.6 (▽) stored in plain glass vials at 25°C.

on the temperature of storage or the pH of the buffer. The rate constants increased as the temperature or pH increased. Estimation of the half-lives of mitoxantrone stored in plain glass vials resulted in lower values than those in Aquasil-treated glass vials.

The degradation of mitoxantrone entrapped in negatively charged liposomes at pH 3.6, 5.8, 7.4 and 9.6 stored in plain glass vials at 25°C is shown in Fig. 3 (results obtained at 4 and 37°C not shown). The degradation of mitoxantrone demonstrated a pseudo-first-order kinetic pattern. At pH 3.6 and 9.6, the residual concentration of mitoxantrone amounted to a value of 100% at zero time of storage. In contrast, at pH 5.8 and 7.4, mitoxantrone resulted in a concentration of about 65% at zero time. About 35% of the initial concentration of mitoxantrone was lost. As mentioned before, this may due to the adsorption of mitoxantrone on the glass surface. In the case of mitoxantrone entrapped in liposomes stored in Aquasil-treated vials, a pseudo-first-order degradation process was also evident (Fig. 4), however, no adsorption of the mitoxantrone was observed.

Tables 3 and 4 show the degradation rate constants of mitoxantrone entrapped in negatively charged liposomes stored in plain and Aquasil-treated glass vials, respectively. It is clear that the rate constants of mitoxantrone in plain vials are greater than those in Aquasil-treated

Table 1
Degradation rate constants (day^{-1}) of mitoxantrone solution stored in plain glass vials

pH	Temperature (°C)		
	4	25	37
3.6	2.57×10^{-3}	3.04×10^{-3}	6.85×10^{-3}
5.8	2.08×10^{-2}	6.52×10^{-2}	1.01×10^{-1}
7.4	5.30×10^{-2}	1.07×10^{-1}	1.38×10^{-1}
9.6	7.00×10^{-2}	1.20×10^{-1}	5.80×10^{-1}

Table 2
Degradation rate constants (day^{-1}) of mitoxantrone solution stored in Aquasil-treated glass vials

pH	Temperature (°C)		
	4	25	37
3.6	2.15×10^{-3}	2.83×10^{-3}	4.65×10^{-3}
5.8	6.31×10^{-3}	4.66×10^{-2}	7.83×10^{-2}
7.4	2.80×10^{-2}	5.03×10^{-2}	1.26×10^{-1}
9.6	4.30×10^{-2}	8.95×10^{-2}	2.26×10^{-1}

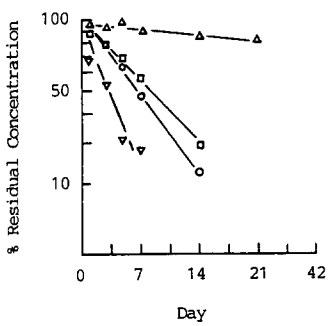

Fig. 4. Concentration-time plots of mitoxantrone entrapped in negatively charged liposomes at pH 3.6 (△), 5.8 (□), 7.4 (○) and 9.6 (▽) stored in Aquasil-treated glass vials at 25°C.

Table 3
Degradation rate constants (day^{-1}) of mitoxantrone entrapped in negatively charged liposomes stored in plain glass vials

pH	Temperature (°C)		
	4	25	37
3.6	5.59×10^{-3}	6.83×10^{-3}	1.06×10^{-2}
5.8	9.59×10^{-3}	5.00×10^{-2}	9.07×10^{-2}
7.4	1.43×10^{-2}	5.29×10^{-2}	9.00×10^{-2}
9.6	1.85×10^{-2}	1.53×10^{-1}	1.73×10^{-1}

glass vials. It was found that the half-lives for mitoxantrone in negatively charged liposomes stored in Aquasil-treated vials were longer than those stored in plain vials. At lower pH and temperature, the half-life was greater. For example, at pH 3.6 and 4°C, the entrapped mitoxantrone in negatively charged liposomes led to a half-life of 223 days as stored in the treated glass vial.

Fig. 5a–d shows plots of the logarithm of the rate constant vs pH for mitoxantrone solution stored in plain vials, stored in Aquasil-treated vials, mitoxantrone entrapped in negatively charged liposomes stored in plain vials, and entrapped in negatively charged liposomes stored in Aquasil-treated vials, respectively. This indicated that the degradation rates of the mitoxantrone in solution and in liposomes increased with increasing pH of the medium. Optimum stability for mitoxantrone was demonstrated to occur at pH 3.6. It is likely that this is a hydroxyl ion catalysis reaction (Connors et al., 1979; Chang, 1990). Also, from Fig. 5a–d, it was found that the rate constants increased as the storage temperature increased. That is to say, at 4°C, mitoxantrone is

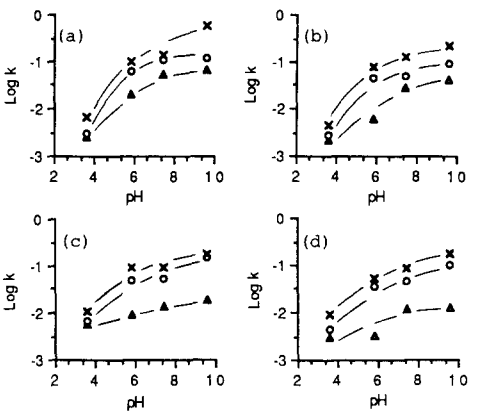

Fig. 5. Logarithm of rate constant against pH plots for mitoxantrone solution stored in plain glass vials (a) and Aquasil-treated glass vials (b), and mitoxantrone entrapped in negatively charged liposomes stored in plain glass vials (c) and Aquasil-treated glass vials (d) at 4 (▲), 25 (○) and 37°C (×).

significantly stable. From the above results, the optimum conditions for the storage of mitoxantrone entrapped in negatively charged liposomes are found to involve Aquasil-coated glass vials at pH 3.6 and 4°C.

Table 5 shows the results of the pseudo-first-order degradation rate constants of mitoxantrone entrapped in negatively charged, positively charged and neutral liposomes stored in Aquasil-treated glass vials at 25°C. The rate constants for the mitoxantrone entrapped in negatively charged, positively charged or neutral liposomes increased with increasing pH of the medium. Generally speaking, there is no significant difference among the rate constants obtained for mitoxantrone entrapped in negatively

Table 4
Degradation rate constants (day^{-1}) of mitoxantrone entrapped in negatively charged liposomes stored in Aquasil-treated glass vials

pH	Temperature (°C)		
	4	25	37
3.6	3.11×10^{-3}	4.34×10^{-3}	9.24×10^{-3}
5.8	3.41×10^{-3}	3.67×10^{-2}	5.45×10^{-2}
7.4	1.15×10^{-2}	4.64×10^{-2}	8.64×10^{-2}
9.6	1.29×10^{-2}	1.01×10^{-1}	1.80×10^{-1}

Table 5
Degradation rate constants (day^{-1}) of mitoxantrone entrapped in negatively charged, positively charged and neutral liposomes stored in Aquasil-treated glass vials at 25°C

pH	Liposome		
	Negatively charged	Positively charged	Neutral
3.6	4.34×10^{-3}	5.01×10^{-3}	5.12×10^{-3}
5.8	3.67×10^{-2}	3.35×10^{-2}	3.00×10^{-2}
7.4	4.64×10^{-2}	5.43×10^{-2}	3.96×10^{-2}
9.6	1.01×10^{-1}	1.34×10^{-1}	1.06×10^{-1}

charged, positively charged or neutral liposomes with the influence of pH, although the rate constant for mitoxantrone in negatively charged liposomes at pH 3.6 was lower than those in positively charged or neutral liposomes. It is suggested that mitoxantrone entrapped in negatively charged, positively charged and neutral liposomes can result in similar stability, except that for negatively charged liposomes at pH 3.6, the mitoxantrone gaining in stability.

Silanization of the glass vials was performed using the aqueous and organic solvent methods with the commercially available reagents of Aquasil and Surfasil, respectively. Table 6 shows the effect of the coating methods on glass vials on the degradation rate constants of the mitoxantrone entrapped in negatively charged liposomes at 25°C. The degradation of mitoxantrone in negatively charged liposomes in both Aquasil- and Surfasil-treated glass vials obeyed pseudo-first-order kinetics. Similar results on the degradation constants of mitoxantrone from the Surfasil- and Aquasil-treated glass vials were obtained. It is expected that comparable values for the half-life can be achieved for the degradation of mitoxantrone as stored in both types of containers.

Ascorbic acid and sodium metabisulfite have been used in mitoxantrone solution and mitoxantrone-containing plasma as an antioxidant. It was shown that the stability of mitoxantrone was greatly enhanced (Reynolds et al., 1981; Peng et al., 1982; Choi et al., 1987; Lin et al., 1989; Chang, 1990; Hu et al., 1990). Fig. 6 depicts the logarithm of concentration remaining vs time profiles for mitoxantrone in negatively charged liposomes stored in Aquasil-treated glass vials with and without the addition of ascorbic acid at pH 3.6 and 5.8, and at 25°C. Apparently, a pseudo-first-order reaction for the degradation of mitoxantrone was demonstrated in the presence and absence of ascorbic acid. The linear relationship and the rate constants obtained for both systems at pH 3.6 and 5.8 exhibited no significant difference. It is likely that the presence of ascorbic acid exhibits no effect on the increase of stability for the entrapped mitoxantrone. This may be due to the ascorbic acid being added in the external phase of the medium which would not affect the oxidation reaction of mitoxantrone taking place inside the liposomes.

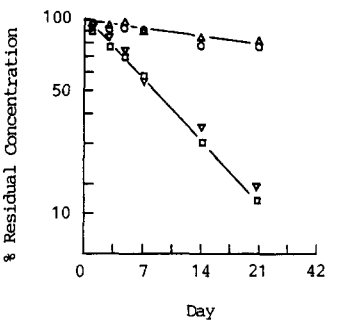

Fig. 6. Concentration-time plots of mitoxantrone entrapped in negatively charged liposomes with and without ascorbic acid stored in Aquasil-treated glass at 25°C. (○) At pH 3.6 with ascorbic acid, (△) at pH 3.6 without ascorbic acid, (▽) at pH 5.8 with ascorbic acid and (□) at pH 5.8 without ascorbic acid.

Table 6
Degradation rate constants (day^{-1}) of mitoxantrone entrapped in negatively charged liposomes stored in Surfasil and Aquasil-treated glass vials at 25°C

pH	Glass vial	
	Surfasil treated	Aquasil treated
3.6	4.29×10^{-3}	4.34×10^{-3}
5.8	3.02×10^{-2}	3.67×10^{-2}
7.4	4.34×10^{-2}	4.64×10^{-2}
9.6	1.08×10^{-1}	1.01×10^{-1}

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