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Effect of various cyclodextrins on solution stability and dissolution rate of doxorubicin hydrochloride

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Summary

A number of cyclodextrins were examined as to their ability to stabilize doxorubicin in solution and to enhance the rate of its dissolution. 2-Hydroxypropyl-β-cyclodextrin (HPβCD), 2-hydroxypropyl-γ-cyclodextrin (HPγCD) and γ-cyclodextrin (γCD) all acted to decrease degradation of doxorubicin in solution although the γ-cyclodextrin derivatives were decidedly more effective. Lineweaver-Burk analysis indicated that the incorporated anthracycline degraded 6–10-times more slowly than did doxorubicin in systems which did not contain cyclodextrin. The chemically modified cyclodextrins were also shown to enhance the rate of dissolution of prototype lyophilized formulations. Thus, HPγCD, present in 5-fold excess relative to doxorubicin, acted to decrease dissolution time from 26 min (for a doxorubicin/lactose dosage form) to less than 9 min. Similarly, HPβCD decreased dissolution time for various prototype formulations. Finally, a complex of HPβCD and doxorubicin was found to be less toxic in a dermal extravasation model. These results, along with the accrued toxicity data which suggest HPβCD is innocuous even when given in large doses parenterally, highly support the use of the modified cyclodextrin as a stabilizing and dissolution-enhancing excipient.

Introduction

Doxorubicin (Dox) is a highly useful antineoplastic agent which is particularly efficacious against both Hodgkin's and non-Hodgkin's lymphoma, carcinoma of the ovary, breast, endometrium and testes, various sarcomas and metastatic thyroid carcinoma (Calabresi and Chabner, 1990). Chemically, the anthracycline is defined as a tetracyclic anthraquinoid aglycone, doxorubicinone, attached through a glycosidic bond to daunosamine, an amino sugar. While several parenteral formulations are available for Dox, a variety of pharmaceutical problems complicate their use. The alkaloid is poorly stable in solution and undergoes acid-catalyzed glycosidic bond hydrolysis, A-ring aromatization subsequent to cleavage of the 9-hydroxymethylketone function and photodecomposition (Tavoloni et al., 1980; Wasserman and Bundgaard, 1983; Beijnen

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et al., 1985, 1986; Janssen et al., 1985; Asker and Habib, 1988). The latter reaction occurs rapidly enough to cause meaningful losses in drug potency after even short exposures to light.

A second problem with intravenous use of Dox is that simple lyophilized preparations dissolve slowly and incompletely upon reconstitution. As such various dissolution-enhancing agents are necessary to allow for convenient dosage from reconstituted preparations. Unfortunately, few parenterally safe agents are currently available to aid in Dox solubilization and stabilization. Addition of methylparaben does decrease the reconstitution time for the lyophilized preparations but does not increase shelf-life of the resulting product. One approach which may be applied in overcoming these limitations involves stabilization by using cyclodextrin and cyclodextrin derivatives.

Cyclodextrins are cyclic oligomaltoses derived from corn starch which have been shown to improve the solution stability and dissolution rate for a number of drugs (Szejtli, 1982; Sekikawa et al., 1983; Chow and Karara, 1986; Duchêne, 1987; Yoshida et al., 1988; Yu et al., 1989). The parent compounds, α-, β-, and γ-cyclodextrin, contain six, seven and eight α-1,4-linked glucose residues, respectively, and exert their beneficial properties by virtue of their ability to form reversible inclusion complexes with drugs (Duchêne, 1987). Unfortunately, β-cyclodextrin, while of appropriate dimensions to efficiently form complexes with a variety of pharmaceuticals, is itself only poorly water soluble (1.85% w/v at 25 °C). This physicochemical limitation imparts to β-cyclodextrin severe renal toxicity when the starch derivative is administered parenterally, precluding its use by this route (Frank et al., 1976; Perrin et al., 1978; Hiasa et al., 1981).

One means of enhancing the aqueous solubility of β-cyclodextrin without perturbing its ability to form inclusion complexes is chemical derivatization. Hydroxyalkylation, for example, gives rise to a complex isomeric mixture which is highly water soluble (> 100% w/v), rapidly dissolving and which lacks significant surface activity (Pitha and Pitha, 1985; Pitha et al., 1986; Pitha, 1988). The latter characteristic provides for a high degree of biological compatibility of the cyclodextrin with membranes and means that agents such as 2-hydroxypropyl-β-cyclodextrin (HPβCD) are not hemolytic or irritating to skeletal muscles, mucous membranes or the eye (Yoshida et al., 1988). Furthermore, the amorphous preparations lack systemic toxicity and are devoid of detrimental effects on the kidney (Brewster et al., 1990; Coussement et al., 1990). Given the parenteral usefulness of hydroxyalkylated cyclodextrins, their reported ability to stabilize and solubilize various drugs and their proven safety by the i.v. route, these agents were examined as possible excipients for improving the pharmaceutical properties of Dox dosage forms.

Materials and Methods

Chemistry

Doxorubicin hydrochloride was obtained from Ben Venue Laboratories, Inc. (Bedford, OH). 2-Hydroxypropyl-β-cyclodextrin (HPβCD) and 2-hydroxypropyl-γ-cyclodextrin (HPγCD) were prepared as previously described (Pitha et al., 1986; Brewster et al., 1990). Briefly, β- or γ-cyclodextrin were solubilized in 4 M sodium hydroxide and then treated with propylene oxide. The reaction was maintained at 0 °C for 2 days and then warmed to room temperature. Subsequent to ion-exchange chromatography (Dowex 50), the crude product was lyophilized, suspended in acetone to remove polypropylene glycols, filtered, dissolved in water and relyophilized. Trace elements, organic and solvent contaminants were then quantitated chromatographically (GC, HPLC or aqueous size exclusion chromatography (ASEC)) or by microcombustion analysis. The average number of hydroxypropyl groups per cyclodextrin nucleus, i.e., the degree of substitution, was determined by fast atom bombardment (FAB) mass spectrometry and was found to be 7.0 in the case of HPβCD and 7.6 in the case of HPγCD. γ-Cyclodextrin was obtained from Sigma Chemical Co. (St. Louis, MO). All other solvents and reagents were of spectral grade and obtained from Aldrich Chemical Co. Deionized water (18.6 Ω) was obtained using a Barnsted Nanopure II Ultrapure Water System. Commercial formula-

tions of Dox were obtained from the manufacturer (Adriamycin RDF from Adria Laboratories and Doxorubicin HCl from Cetus, Inc.).

Solid complex preparation

Several complexes were generated which contained various proportions of either HPβCD or HPγCD and Dox. In these samples, Dox (8 mg/ml) was formulated in a 1 ml aqueous solution containing one of the following: 40, 80 or 160 mg/ml HPβCD, 40 mg/ml HPγCD or 40 mg/ml lactose. In the case of the 40 mg/ml HPβCD system, a sample was also prepared in which 0.001 N hydrochloric acid was used in place of the 1 ml of water. Each vial was then lyophilized to provide a solid for which the Dox component was quantitated as described below. The above manipulations produce complexes of HPβCD containing 17, 9.1 and 4.7% Dox, HPβCD complexes containing 17% Dox and a mixture of lactose with Dox which contained 17% Dox, respectively (w/w).

Analytical methodology

Dox was quantitated by HPLC using a modification of a compendial method (USP XXII, 1990). The system configuration included a Spectra-Physics (SP8800) ternary solvent pump, an SP8500 dynamic mixer, an SP8780 refrigerated autosampler, an SP8490 variable UV/visible wavelength detector, and an SP4270 integrator networked, using Labnet software, to an IBM microprocessor. Separation was effected on an Alltech Absorbosphere HS C18 5 μm particle size column (25 cm × 4.6 mm i.d.). The mobile phase contained acetonitrile : 0.02 M phosphoric acid with 0.01 M sodium lauryl sulfate 60 : 40 and the flow rate was 1.5 ml/min. Assays were standardized internally with *n*-hexyl *p*-hydroxybenzoate. At ambient temperature, the above-described system produced retention times of 2.7 and 8.4 min for Dox and the internal standard, respectively.

Kinetic evaluation

The stability of Dox was examined in a variety of solutions in which the pH, buffer species, ionic strength, as well as cyclodextrin type, and concentration were manipulated. In these studies, a small volume (20 μl) of a methanolic Dox solution was added to 2 ml of the test matrix equilibrated to 75 °C so that the initial drug concentration was 6×10^{-5} M. Test matrices which were designed to probe the effect of pH and buffer salt type and concentration included hydrochloric acid (0.01 M, pH 1.88; 0.05 M, pH 1.88; 0.10 M, pH 1.01), formate buffer (0.90 M, pH 2.52), acetate buffer (0.42 M, pH 3.44; 0.80 M, pH 4.52), phosphate buffer (0.44 M, pH 5.90; 0.14, 0.09, 0.07 and 0.04 M, all adjusted to pH 7.42; 0.20 M, pH 7.72), borate buffer (0.08 M, pH 7.82) and Tris buffer (0.10 M, pH 8.30). In the above systems, ionic strength was controlled with sodium chloride and maintained at $\mu = 0.5$. In a second series of experiments, solutions of hydrochloric acid (0.01 M, pH 1.88; 0.10 M, pH 1.01) were adjusted to ionic strengths between 0.1 and 0.5 with sodium chloride. Finally, the effect of 5% w/v HPβCD, HPγCD and γ-CD on Dox stability was evaluated in two media at four pH values including aqueous hydrochloric acid (0.10 M, pH 1.01; 0.01 M, pH 1.84) and phosphate buffer (0.44 M, pH 5.90; 0.20 M, pH 7.72).

While the above-described studies were conducted at 75 °C, a second group of experiments were evaluated at room temperature. In these circumstances, Dox was dissolved in aqueous solutions of lactose, HPβCD, HPγCD, saline or various combinations of the excipients. Two concentrations of Dox were used in the prototype formulations (0.2 and 2.0 mg/ml). The 0.2 mg/ml samples were stored in 2 ml clear glass vials (Opticlear, Kimble) which were closed with septa and secured with aluminium seals while the 2.0 mg/ml samples were contained in 7.0 ml clear glass vials (SolventSaver Scintillation Vials, Kimble) which were closed with teflon-lined screw caps. All vials were then stored at room temperature (22 ± 2 °C) on a glass shelf exposed to light at an average intensity of 305 footcandles (Sylvania fluorescent light tubes, No. F15T8-D) for 24 days. At various times, four (0.2 mg/ml) or three (2.0 mg/ml) samples were removed and assayed by HPLC and values averaged.

In all kinetic studies, reactions were performed under pseudofirst-order conditions. The observed rate constant (k_{obs}) was determined by

linear regression analysis of the natural logarithm of the chromatographic peak areas for Dox as a function of time. Half-lives ($t_{1/2}$) were obtained based on the relationship $t_{1/2} = 0.693/k_{obs}$. Stability constants for cyclodextrin interaction with Dox (1:1 complexation) as well as degradation constants for Dox included in the cyclodextrin cavity (k_c) were determined using Lineweaver-Burk analysis (Bekers et al., 1988). This procedure used the following equation:

$$k_0/(k_0 - k_{obs}) = k_0/\{K_c(k_0 - k_c)[CD]\} + k_0/(k_0 - k_c)$$

where k_0 is the first-order degradation rate of Dox in the absence of cyclodextrins. k_{obs} denotes the rate of Dox disappearance in solutions containing cyclodextrins and [CD] is the cyclodextrin concentration at which the experiment was carried out. Using this relationship, k_c and K_c can be calculated from the intercept and slope, respectively, of the straight line obtained from a plot of $k_0/(k_0 - k_{obs})$ vs $1/[CD]$.

Dissolution studies

The rate of dissolution of prepared solid complexes of Dox and either HPβCD or HPγCD was determined. In the paradigm employed, freeze-dried samples of Dox and HPγCD (8 mg Dox/48 mg total), Dox and HPβCD (8 mg Dox/48 mg, 8 mg Dox/88 mg or 8 mg Dox/168 mg total) and physical mixtures of Dox and lactose were accurately weighed into 20 ml glass vials. The amount of complex aliquoted was adjusted so that subsequent to reconstitution, the concentration or Dox was maintained at 2.0 mg/ml. The vials containing the dry powder were positioned in a shaking water bath (Model YB-521, American Scientific Products), maintained at a temperature of 22.4 ± 0.5 °C and were equilibrated at a rate of 62 oscillations/min. At time zero, 2.0 ml of normal saline were added to the vials. Samples (50 μl) were then removed by reverse filtration at various times and the withdrawn aliquots diluted in 50% aqueous methanol. Samples were analyzed by HPLC for appearance of Dox with three to six replicates examined for each formulation. Dissolution times were calculated by fitting average Dox concentration values expressed either as normalized peak heights of percent dissolved to the following first-order model:

$$a = ai(1 - e^{-kt})$$

where a is the average peak height, t represents time (in min), k is the rate constant and ai is a maximum dissolution parameter. The half-life for dissolution was calculated as before and the dissolution time was estimated at 90% of the ai which is approximately equal to four half-lives. In all cases, rate constant estimates were obtained with the aid of the MINSQ (Version 3.12, Micromath, Inc.) software.

Results and Discussion

The stability of Dox in aqueous solutions of various compositions was examined. In all cases, Dox degraded in a first-order fashion which was linear over at least four half-lives. Table 1 illustrates the effect of pH and buffer species on stability at constant ionic strength ($\mu = 0.5$) and temperature (75 °C). The anthracycline was most stable at pH values between 3 and 4 in agreement

TABLE 1

Experimental conditions and the pseudofirst-order rate constants (k_{obs}) for the overall loss of doxorubicin from aqueous buffer solutions at 75 °C [ionic strength μ 0.5 (NaCl)]

pH	Buffer and total concentration	k_{obs} (min^{-1})
1.01	0.10 M hydrochloric acid	0.173
1.18	0.05 M hydrochloric acid	0.111
1.88	0.01 M hydrochloric acid	2.18×10^{-2}
2.52	0.90 M formate buffer	2.80×10^{-3}
3.44	0.42 M acetate buffer	2.32×10^{-3}
4.52	0.80 M acetate buffer	3.77×10^{-3}
5.90	0.44 M phosphate buffer	1.23×10^{-2}
7.42	0.14 M phosphate buffer	4.24×10^{-2}
7.42	0.09 M phosphate buffer	3.21×10^{-2}
7.42	0.07 M phosphate buffer	3.05×10^{-2}
7.42	0.04 M phosphate buffer	2.80×10^{-2}
7.72	0.20 M phosphate buffer	5.48×10^{-2}
7.82	0.08 M borate buffer	7.37×10^{-2}
8.30	0.10 M Tris buffer	0.156

TABLE 2

Effect of ionic strength on the rate of degradation of doxorubicin in aqueous hydrochloric acid solutions at 75.0 °C

pH	Ionic strength (μ)	$\sqrt{\mu}$	k_{obs} (min^{-1})
1.01	0.10	0.32	9.92×10^{-2}
1.01	0.20	0.45	0.127
1.01	0.30	0.55	0.153
1.01	0.50	0.71	0.173
1.88	0.20	0.45	1.17×10^{-2}
1.88	0.30	0.55	1.67×10^{-2}
1.88	0.50	0.71	2.17×10^{-2}

The ionic strength was adjusted by addition of NaCl to the reaction medium.

TABLE 3

First-order rate constants (k_{obs}) for the degradation of doxorubicin in aqueous buffer solutions containing no cyclodextrin or 5% (w/v) hydroxypropyl-β-cyclodextrin (HPβCD), γ-cyclodextrin (γ-CD) or 2-hydroxypropyl-γ-cyclodextrin (HPγCD) at 75.0 °C

pH	$k_{obs}(\times10^{-2})(min^{-1})$			
	No cyclodextrin	HPβCD	γ-CD	HPγCD
1.01	17.3	14.4	7.16	8.26
1.84	1.86	1.26	1.39	0.72
5.90	1.23	1.01	0.71	0.57
7.72	5.48	2.68	4.52	2.13

with earlier work (Beijnen et al., 1985). At lower pH values, the glycosidic bond cleaves while at higher pH values, a complex series of reactions occurs only some of which have been established. A product which does form at pH > 4 is one in which the A-ring aromatizes and undergoes 9-α-side chain loss. In addition, buffer catalysis was observed in the case of various phosphate ions at pH 7.4.

The effect of varying ionic strength on Dox degradation in dilute hydrochloric acid (pH 1.01 and 1.88) is provided in Table 2. As shown, there is a clear effect of altering sodium chloride concentration as increasing ionic strength from μ = 0.1 to μ = 0.5 enhances Dox disappearance. Plots of $\sqrt{\mu}$ vs log k_{obs} were found to be highly linear, consistent with earlier findings (Beijnen et al., 1985), which suggested that the degradation of Dox at low pH involved the formation of a polar transition state.

The effect of various cyclodextrins on Dox stability at constant ionic strength (μ = 0.5) and temperature (75 °C) is demonstrated by the data in Table 3. In these studies, HPγCD, HPβCD and γCD were maintained at 5% w/v in the test matrices and initial Dox concentration at 6×10^{-5} M. The rate constant data indicate that all three cyclodextrins had a stabilizing effect. The β-cyclodextrin derivative, however, was less effective in this regard than was either γ-CD or HPγCD. HPβCD reduced the rate of Dox degradation by 17% at pH 1.01, 32% at pH 1.84, 18% at pH 5.9 and by more than half (51.1%) at pH 7.72. γ-CD was more effective at all pH conditions except 7.72 and HPγCD induced rate decelerations of 52, 61, 53 and 61% at pH values of 1.01, 1.84, 5.90 and 7.72, respectively. The HPγCD mixture was thus shown to be the most useful stabilizing agent increasing half-lives by between 2- and 2.6-fold. The greater interaction between Dox and γ-CD prompted a more detailed study of the effect of HPγCD on Dox stability. To this end, Lineweaver-Burk analysis was completed for the degradation of Dox in the presence of HPγCD (Table 4; Figs 1 and 2) (Bekers et al., 1988). These evaluations showed that the 1:1 equilibrium stability constant (K_c) reached a maximum at a pH of 6. Importantly, Dox was found to degrade significantly slower when included in the cyclodextrin cavity than

TABLE 4

Influence of HPγCD on the degradation of doxorubicin at various pH values and 75.0 °C

pH	$k_0(\times10^{-3})$ (min^{-1})	$k_c(\times10^{-3})$ (min^{-1}) [k_0/k_c]	K_c (M^{-1})	r
1.01	173	30.2 [5.7]	69.78	0.99
1.84	18.6	2.1 [8.9]	193.1	0.99
5.90	12.3	4.7 [2.6]	243.3	0.99
7.72	54.8	10.3 [5.3]	132.3	0.99

k_0, rate constant for degradation of free drug; k_c, rate constant for degradation of drug within the complex; K_c, stability constant for the complex; r, correlation coefficient for Lineweaver-Burk linear line fit (see text for details).

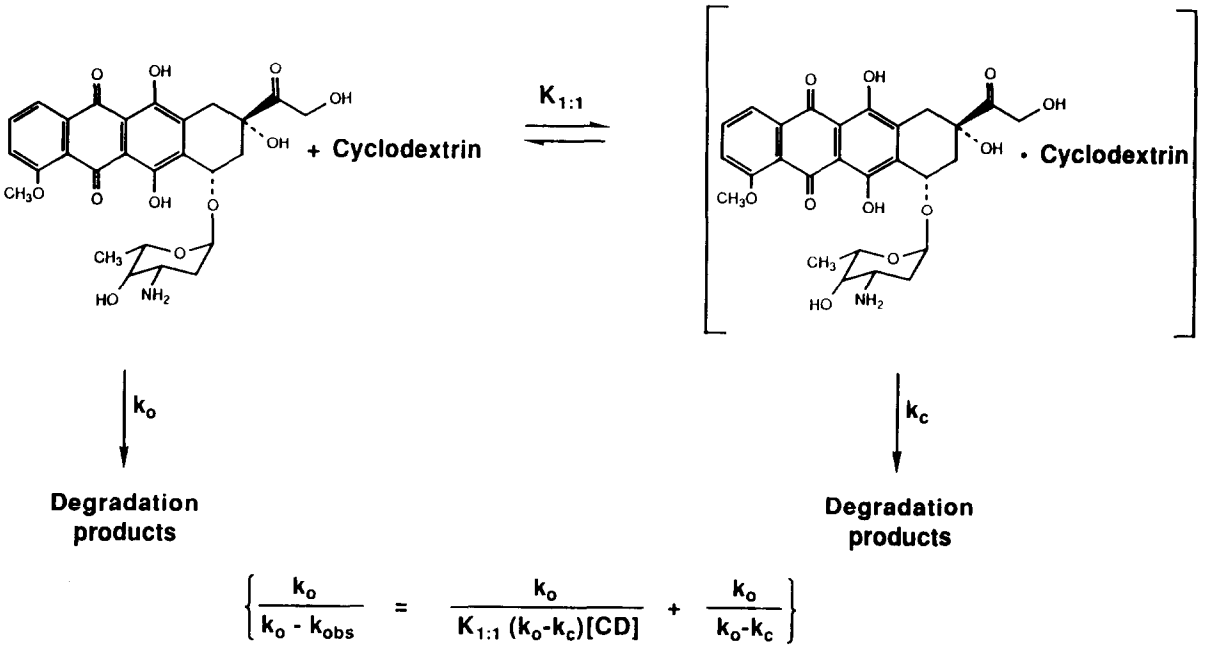

Fig. 1. Lineweaver-Burk analysis for decomposition of Dox in the presence or absence of various cyclodextrins.

outside this enclosure. The stabilizing effect was maximal at pH 1.8 (9-fold).

The stabilizing effect of cyclodextrins on other polycyclics has been similarly observed. γ-Cyclodextrin is known to inhibit photodecomposition of phenothiazines, oxidation of chlorpromazine and dehydration of prostaglandins. (Pagington, 1987; Uekama, 1987; Uekama and Otagiri, 1987). Uekama has also demonstrated that cyclodextrins can inhibit glycoside decomposition as in the case of digitoxin (Uekama et al., 1982; Yoshida et al., 1988b). Chemically modified cyclodextrins including HPβCD were found to significantly slow sugar cleavage in the cardiac glycoside and were especially useful in inhibiting transformation of the monoglycoside (monodigitoxose) to the aglycone (digitoxigenin). β-Cyclodextrin provided for a 100-fold reduction in this rate constant while dimethyl-β-cyclodextrin lowered this rate by 2400-fold (Yoshida et al., 1988b). Bekers et al. (1988) also found that γCD slowed Dox glycosidic bond cleavage by over 5-fold at pH 1.5 (50 °C, $\mu = 0.3$).

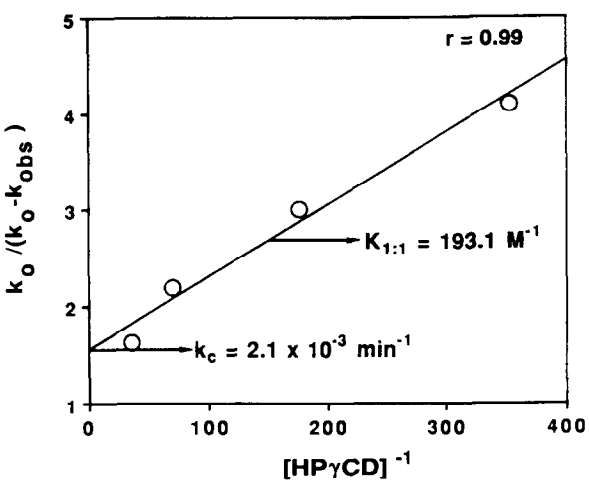

Fig. 2. Effect of 2-hydroxypropyl-γ-cyclodextrin (HPγCD) on the rate of degradation of Dox at pH 1.84. The above Lineweaver-Burk analysis allows for the determination of the complex stability constant ($K_{1:1}$) as well as the rate constant for Dox degradation when incorporated into cyclodextrin (k_c).

Room-temperature stability studies are summarized in Table 5. Low concentrations of both HPγCD and HPβCD (1 mg/ml) appeared to have little effect on Dox in these systems which were unbuffered and unprotected from light. At more concentrated levels (10–200 mg/ml), both

modified cyclodextrins had a significant effect on stability. The data suggest, therefore, that replacement of lactose with either HPβCD or HPγCD could yield a formulation which was stable (t_{90}) for almost 5 days. The simple lactose reconstitution degraded by 10% within 2 days.

Dissolution rates of prototype formulations or commercially available dosage forms were completed by following a modified literature procedure (Nogami et al., 1969). In this method, the appearance of Dox in solution was fitted to a first-order kinetic model, the results of which are given in Table 6 and Fig. 3. A simple lactose/Dox formulation reconstituted with normal saline required 26 min to dissolve. Replacement of the lactose with either HPγCD or HPβCD significantly accelerated this process. Thus, a solid containing 4.7% w/w Dox incorporated into HPβCD was found to dissolve in less than half the time

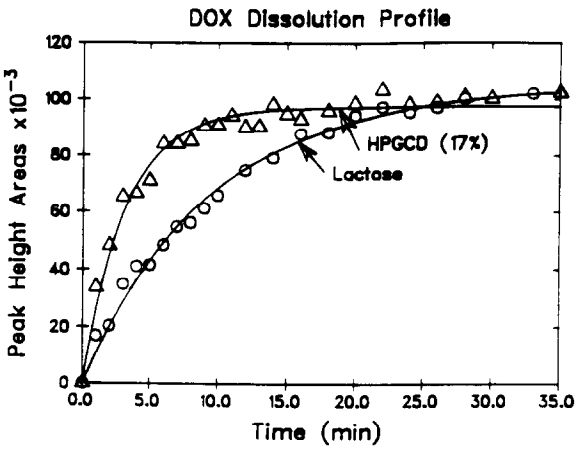

Fig. 3. Rate of dissolution for a commercially obtained lactose-containing Dox formulation and a prototype dosage form containing 17% Dox in hydroxypropyl-γ-cyclodextrin (HPγCD). Kinetics were obtained using the MINSQ® software package.

TABLE 5

Experimental conditions, pseudofirst-order rate constants (k_{obs}), half-lives (t_{50}), shelf-lives (t_{90}) and correlation coefficients for the overall loss of doxorubicin from unbuffered aqueous solutions at 22.4 ± 0.5 (S.D.)° C

Concentration (mg/ml)					pH	k_{obs} ($\times 10^2$) (days)	$t_{1/2}$ (days)	t_{90} (days)	Correlation coefficient
Doxorubicin HCl	Lactose	HPβCD	HPγCD	NaCl					
0.2	1.0	0.0	0.0	9.0	5.73	6.35	10.9	1.7	0.974
0.2	0.0	1.0	0.0	9.0	6.36	8.04	8.6	1.3	0.985
0.2	0.0	0.0	1.0	9.0	4.74	6.76	10.3	1.6	0.995
2.0	0.0	0.0	10.0	9.0	5.18	3.49	19.9	3.0	0.906
2.0	0.0	200	0.0	0.0	5.10	2.65	26.2	4.0	0.989
2.0	0.0	0.0	200	0.0	4.95	2.25	30.8	4.7	0.884

In this study, light intensity was maintained at 305 footcandles.

TABLE 6

Rate constants (k) for dissolution with 95% confidence intervals, half-lives, dissolution time and correlation coefficients for a variety of Dox (2 mg/ml) formulations containing lactose, lactose and methyl paraben or various cyclodextrins

Formulations	k (±95% confidence intervals)	$t_{1/2}$ (min)	Dissolution time (min)	Correlation coefficient
Lactose/Dox	0.11 ± 0.01	6.50	26.3	0.996
Paraben/Dox	0.39 ± 0.13	1.77	7.1	0.940
HPγCD/Dox (17%)	0.31 ± 0.04	2.24	8.9	0.989
HPβCD/Dox (17%)	0.18 ± 0.02	3.85	15.4	0.986
HPβCD/Dox (9%)	0.22 ± 0.04	3.15	12.6	0.978
HPβCD/Dox (4.7%)	0.25 ± 0.06	2.76	11.0	0.952
HPβCD/Dox (17%) with HCl	0.09 ± 0.02	8.06	32.2	0.981

(11.1 min) of the lactose-based system. Increasing the Dox concentration in the solid to 9% gave a cake which behaved similarly with 12.6 min required for dissolution. Further increases in the rate of dissolution were observed in a HPγCD complex which contained 17% w/w Dox which was found to dissolve in less than 9 min. The latter formulation compared favorably in its dissolution kinetics to a commercially available paraben-containing rapidly dissolving formulation which, in the above-described assay, gave a dissolution time of 7.5 min.

A second approach for evaluating the dissolution-enhancing properties of HPγCD and HPβCD involved simple observation subsequent to repeated episodes (10 s) of shaking the prototype dosage forms. The results of this subjective assay are listed in Table 7. The dosage forms behaved substantially the same in this assay as in the HPLC evaluation described earlier. The results indicated that the simple lactose/saline reconstitution required 11 repeated 10-s agitation cycles to provide complete dissolution while only two 10-s periods were required for the HPβCD/Dox system (4.7% Dox).

The enhanced rate of dissolution offered by cyclodextrins has been extensively described. Importantly, for drugs whose intestinal uptake is limited by the solubility of the agent, such manipulations can lead to increased oral bioavailability. Such improvements have been documented for ibuprofen, prednisolone, dicoumarol, phenytoin, spironolactone and many others (Tsuruoka et al., 1981; Seikawa et al., 1983; Seo et al., 1983; Chow and Karara, 1986; Fukuda et al., 1986; Gandhi and Karara, 1988). In this case of Dox, while enhanced dissolution was sought for pharmaceutical purposes, similarly useful effects were generated by cyclodextrins.

The above-detailed results suggest that HPγCD has a significant stabilizing effect on Dox in solution and that both HPγCD and HPβCD enhance the rate of dissolution for lyophilized formulations intended for parenteral administration. Extensive toxicity assessments have demonstrated that HPβCD is innocuous when introduced i.v., even in large doses. Thus, no significant acute untoward effects were observed in monkeys when HPβCD in i.v. doses as large at 10 g/kg were given (Brewster et al., 1990). Furthermore, human studies involving parenteral HPβCD have all indicated that in single doses as large as 5 g, no adverse reactions occurred and that the excipient was well tolerated (Brewster and Bodor, 1990; Szathmary et al., 1990; Seiler et al., 1990).

TABLE 7

Comparative subjective dissolution rates of prototype formulations containing 2 mg / ml doxorubicin

No. of shakes (10 s each) [a]	Formulation [Total sample weight]					
	Lactose/ Dox [10 mg]	Paraben/ Dox [10 mg]	HPγCD/ Dox (17%) [60.25 mg]	HPβCD/ Dox (17%) [60.39 mg]	HPβCD/ Dox (9%) [110.01 mg]	HPβCD/ Dox (4.7%) [210.5 mg]
1	+ + +	+ + +	+ + +	+ + +	+ + +	+
2	+ + +	0	+ + +	+ + +	+ +	0
3	+ +		+ +	+ +	+	
4	+ +		+ +	+ +	+	
5	+ +		+ +	+ +	0	
6	+		+	+		
7	+		+	+		
8	+		0	+		
9	+			0		
10	+					
11	0					

[a] 5 ml normal saline was added to each preparation and samples were manually shaken for 10 s and then examined. Observations are reported. + + +, globules present; + +, globules present but dispersed; +, few droplets present; 0, dissolved.

Another advantage of the use of modified cyclodextrins in parenteral dosage forms of Dox is the possibility for decreasing extravasation toxicity. Dox is associated with severe skin lesions which occur when a portion of an administered dose seeps into tissue surrounding the venipuncture site. Extravasation toxicity for Dox occurs with an incidence of 0.5–6% and is associated with focal epidermal necrosis, inflammation and extreme pain (Barlock et al., 1979; Ignoffo and Friedman, 1980; Larson, 1982; Auerbach et al., 1988). In many circumstances, surgery is required to replace the area of skin affected. While subsequent administration of glucocorticoids has been suggested to alleviate Dox-associated dermal toxicity, no therapy has been shown to be fully effective. Cyclodextrins have been shown to decrease the toxicity of agents when administered i.m. Yoshida et al. (1990) found that incorporation of the calcium channel blocker, nimodipine, in HPβCD decreased the muscle irritation of this agent by 55%. In addition, muscle toxicity of chlorpromazine was significantly reduced when the phenothiazine was incorporated into cyclodextrin (Uekama and Otagiri, 1987).

Preliminary findings with Dox and HPβCD point to a decrease in extravasation toxicity for the complex compared to the uncomplexed anthracycline. In these studies, 1.0 mg of Dox was administered in 0.5 ml of saline intradermally to male rats. In addition, a complex of Dox and HPβCD was similarly administered so that 1.0 mg of Dox was injected in the same volume of vehicle (Simpkins and Bodor, unpublished data). A third treatment included administration of Dox (1.0 mg in 0.5 ml saline) followed by a second injection of 0.5 ml of a 50% w/v HPβCD aqueous solution. All three treatments produced skin lesions whose size was quantitated by caliper measurements at day 7. Dox in saline produced an area of necrosis 10.3 ± 1.2 mm in diameter. The Dox/HPβCD complex produced a lesion which was significantly smaller by 32% at day 7 ($p < 0.05$, paired comparison). Administration of HPβCD subsequent to Dox dosing did not provide for a statistically smaller wound size.

One final point involves the practicality of administration of the cyclodextrin-containing formulations herein described. The total dose of HPγCD or HPβCD can be estimated based on the maximum tolerable dose of Dox which is 550 mg/m^2. Given a 70 kg, 185 cm individual, the total dose of Dox would be estimated at 1.05 g. Assuming a 2 mg/ml Dox dosage form, the range of HPγCD administered over a chemotherapeutic course would be 5–10 g (10–20 mg/ml in the formulation) and for HPβCD 10–20 g (20–40 mg/ml in the formulation). Results from toxicity studies already completed would support the cumulative dose of the excipient suggested.

In summary, modified cyclodextrins including HPβCD and HPγCD were found to stabilize Dox to varying extents in aqueous solutions maintained at 75 °C. The γ-derivatives appeared to be more effective in this regard than did HPβCD, due presumably to a better fit of Dox into the larger cyclodextrin cavity afforded by the glucose octomer. Room-temperature degradation evaluations also indicated that significant stabilization could be achieved by incorporating either HPβCD or HPγCD into the dosage form. Slow dissolution of Dox formulations is a problem in dose preparation and administration. Both HPβCD and HPγCD were shown to accelerate the rate of dissolution of lyophilized prototype formulations containing these excipients relative to marketed Dox/lactose systems. Finally, initial results using a rat model for extravasation toxicity indicate that HPβCD significantly reduces Dox-associated skin lesions. All of these observations in combination with the parenteral safety of examined hydroxyalkyl cyclodextrins support their use as stabilizing and solubilizing excipients.

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