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Effects of cyclodextrins on *N*-trifluoroacetyldoxorubicin-14-valerate (AD-32) stability and solubility in aqueous media

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Summary

The effects of cyclodextrins on the chemical stability of *N*-trifluoroacetyldoxorubicin-14-valerate (AD-32) have been investigated using a stability-indicating HPLC assay. The influences of various parameters, such as structure of the cyclodextrin, cyclodextrin concentration and pH, were studied. A phase solubility study of AD-32 with different cyclodextrins has been performed and it was found that all cyclodextrins resulted in an A-type phase-solubility diagram, whereas hydroxypropyl-β-cyclodextrin has the largest solubilizing effect.

Introduction

N-Trifluoroacetyldoxorubicin-14-valerate (AD-32) is a derivative of doxorubicin, reported to display greater antitumour activity in experimental in vitro systems as well as in animals compared to the parent compound (Vecchi et al., 1978; Niell et al., 1986). AD-32 differs from doxorubicin in the presence of a valerate moiety at C14 and a trifluoroacetyl group linked to the 3′-amino group in the sugar moiety (Fig. 1). The physicochemical and biological characteristics of this compound are, therefore, completely different from those of doxorubicin; AD-32 is almost insoluble in water, whereas the mode of cytotoxic action is also totally different (Israel et al., 1980; Arcamone, 1981). The poor solubility hinders the formulation of the drug for pharmaceutical purposes. Anthracyclines are, like other glycosidic antibiotics, susceptible to hydrolytic degradation in aqueous solutions (Beijnen et al., 1985, 1986a). The stability can be increased by using cosolvents (Davignon and Cradock, 1984; Bekers et al., 1989), trapping the drug into micelles or liposomes (Janssen et al., 1985; Bekers et al., 1989) or complexing with a suitable compound.

Cyclodextrins (CyD) (Fig. 2) are known to form inclusion complexes with a number of molecules (Uekama and Otagiri, 1987; Szejtli, 1988). En-

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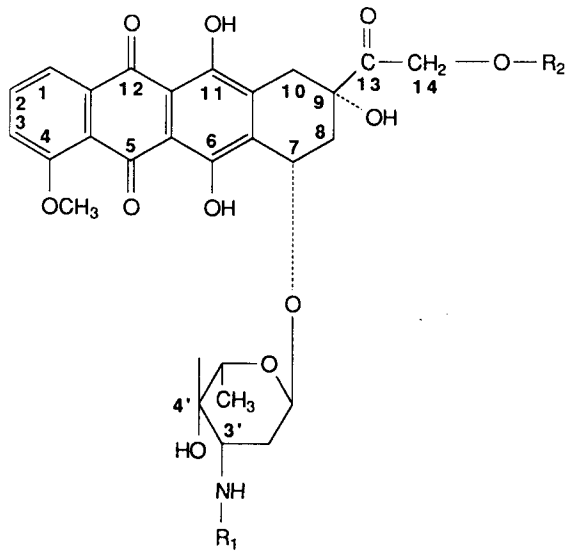

Fig. 1. Structures of the anthracycline antibiotics doxorubicin and AD-32.

	R_1	R_2
Doxorubicin	H	H
AD-32	$COCF_3$	COC_4H_9

capsulation of a molecule may affect many of its physicochemical properties, including chemical stability and aqueous solubility (Jones et al., 1984; Szejtli, 1987). The aim of the present study was to investigate systematically the effects of various CyD on the chemical stability as well as the solubility of AD-32 in aqueous media. Besides the natural CyD, the effects of hydroxypropyl-β-CyD (HP-β-CyD), a semi-synthetic CyD, have been studied, since HP-β-CyD appears to be suitable as a complexing agent in parenteral formulations, due to its low toxicity and high aqueous solubility (Yoshida et al., 1988; Brewster et al., 1989).

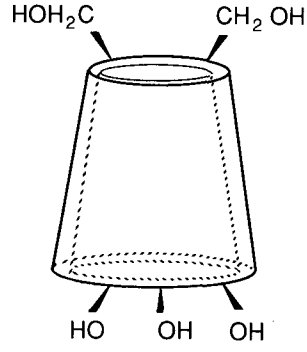

Fig. 2. Schematic representation of a natural cyclodextrin molecule.

Materials and Methods

Chemicals

The natural CyD (α-, β- and γ-CyD) came from Nihon Kako Co. Ltd (Tokyo, Japan) and HP-β-CyD was kindly provided by Dr J. Mesens (Janssen Pharmaceutica, Beerse, Belgium). All CyD were used as received. AD-32 was a gift from Dr S. Penco (Farmitalia, Milan, Italy). All other chemicals used were of analytical grade. Deionized water was filtered through a Milli-Q Water System (Millipore, Bedford, MA, U.S.A.) before use.

Glassware

Because AD-32 has a great tendency to adsorb to all kinds of materials (Tomlinson and Malspeis, 1982), all glassware containing solutions of this compound were silanized before use, employing a solution of trichloromethylsilane in toluene (3%, v/v), and subsequently rinsing with methanol.

Degradation media

The kinetic studies were carried out after dissolving the appropriate amount of the drug in a buffer/CyD solution. The buffer solutions used were: pH 1–3, perchloric acid; pH 3–5, 0.01 M acetate buffer; pH 5–8, 0.01 M phosphate buffer, pH 8–10, 0.01 M carbonate buffer. pH values were measured using a gel-filled combination electrode (Beckman, Fullerton, CA, U.S.A.) and a pH meter (Metrohm, E512, Herisau, Switzerland). Since traces of metal impurities may catalyze the degradation of AD-32, sodium edetate (5×10^{-4} M) was added to the solutions: this chelating agent eliminates the catalyzing effect. A constant ionic strength ($\mu = 0.3$) was maintained by addition of appropriate amounts of sodium chloride.

Kinetic studies

All kinetic experiments in buffer/CyD solutions were performed over at least three half lives.

The solutions were stored in a thermostatically controlled water bath at 50 ± 0.2° C and were protected from light. The reactions were initiated by adding 80 µl of an AD-32 stock solution in methanol (1 mg/ml) to 4 ml of pre-heated buffer/CyD solution. At certain time intervals samples were withdrawn and analyzed immediately with a stability-indicating high-performance liquid chromatographic (HPLC) method, described earlier (Bekers et al., 1989). Samples of partly degraded AD-32 test solutions with $t_{1/2}$ values < 8 min were, to stop the degradation process, immediately mixed with the mobile phase used in the HPLC (1 : 1). These samples are stable for at least 1 week when stored at 4° C and protected from light.

Solubility study

1 mg amounts of AD-32 were put into 25 ml glass-stoppered volumetric flasks containing 5 ml of 0.01 M acetate buffer, pH 4. Various amounts of CyD were added and the flasks were placed in a shaking water bath at 25 ± 0.2° C.

To investigate the dissolution rate of AD-32, samples were taken at appropriate time intervals and analyzed for the content of AD-32. Throughout this study the CyD concentration was 0.12 M. For the investigation of the influence of CyD concentration on the quantity of drug dissolved, samples were analyzed for the content of AD-32 5 days after the start of the experiments. Aliquots of the clear supernatant were taken after centrifugation of the vials, and analyzed by HPLC.

Results and Discussion

Glassware

When CyD were present in aqueous buffer solutions in a concentration of at least 4×10^{-3} M it was found that the adsorption of AD-32 on the glass wall of the vials was negligible, making silanization superfluous.

Degradation kinetics and mechanism

The degradation of AD-32 in the presence of different cosolvents follows a pseudo-first order kinetic pattern (Bekers et al., 1989). It appears that in the presence of CyD the kinetic behaviour, over the whole pH range investigated, remains pseudo-first order. This is indicated by the linearity ($r > 0.999$) of plots of the natural logarithm of the residual AD-32 concentration vs time. From the slopes of these lines the values for the pseudo-first order rate constants, k_{obs} were calculated.

Because CyD has no influence on the kinetic behaviour and the presence of CyD does not alter the kind of degradation products formed, it is concluded that the degradation mechanism for AD-32 in the presence of CyD is identical to that for the drug in the absence of CyD (Bekers et al., 1989).

Standard deviation in k_{obs}

The standard deviation in k_{obs} was determined at pH 1 in the presence of 3.7×10^{-2} M HP-β-CyD. The value of k_{obs} and the standard deviation, calculated from six observations, is $2.6 \pm 0.2 \times 10^{-4}$ s^{-1}. Other rate constants were determined in duplicate.

Influence of various CyD

The influences of various CyD on the degradation rate of AD-32 have been investigated at four pH values; the results are presented in Table 1. It is obvious that at each pH value the effect, if any, of all CyD on the stability of AD-32 must be of the same order of magnitude.

Due to the poor solubility of AD-32 in water k_{obs} values in this medium are not available, and no definite conclusions about a possible effect of CyD on AD-32 stability can be drawn. However, the observed rate constants reported in Table 1 are, in general, of the same order of magnitude as

TABLE 1

Influences of several CyD ([CyD] = 3.7×10^{-2} M) on the degradation of AD-32 at different pH values

CyD	k_{obs} (s^{-1})			
	pH 1	pH 4	pH 7	pH 9
α	3.7×10^{-4}	1.4×10^{-6}	1.5×10^{-5}	4.5×10^{-4}
β	1.2×10^{-4}	4.2×10^{-7}	4.9×10^{-6}	1.5×10^{-4}
HP-β	2.6×10^{-4}	9.2×10^{-7}	1.4×10^{-5}	2.6×10^{-4}
γ	1.1×10^{-4}	3.5×10^{-7}	2.7×10^{-5}	1.3×10^{-3}

TABLE 2

Influence of the γ-CyD concentration on AD-32 degradation at pH 1 and pH 9

[γ-CyD] (M)	k_{obs} (s^{-1})	
	pH 1	pH 9
1.2×10^{-2}	5.3×10^{-5}	6.6×10^{-4}
1.5×10^{-2}	6.2×10^{-5}	7.3×10^{-4}
3.7×10^{-2}	1.1×10^{-4}	1.3×10^{-3}
5.4×10^{-2}	2.2×10^{-4}	2.0×10^{-3}

k_{obs} values determined in a previous study concerning the degradation of AD-32 in water/acetonitrile (1 : 1, v/v) systems (Bekers et al., 1989). Because it is well known that organic modifiers, like acetonitrile, have a stabilizing effect on the degradation of antitumour agents (Davignon and Cradock, 1984; Bekers et al., 1989)), it may be assumed that CyD lead to the same effect.

Influence of CyD concentration

The influence of the CyD concentration on the reaction rate constant of AD-32 has been studied with γ-CyD in acidic and alkaline media. Increasing the γ-CyD concentration leads to a decrease in AD-32 stability. This is documented in Table 2. In alkaline medium the acceleration is in accordance with earlier results concerning the degradation of anthracycline antibiotics in aqueous alkaline solutions (Bekers et al., 1990). However, in acidic solutions doxorubicin, the parent compound of AD-32, and some analogues are stabilized by addition of γ-CyD (Bekers et al., 1988).

An explanation for the stabilization of doxorubicin against the acceleration of AD-32 degradation in acidic medium by γ-CyD can possibly be found in the presence of the valerate moiety at C14 and the trifluoroacetyl group on the 3′-amino function of the sugar moiety (Fig. 1).

The pK_a value of the amino group in doxorubicin is around 8 (Beijnen et al., 1986b); this means that in acidic media doxorubicin is protonated while AD-32 is uncharged. However, no evidence for different complexation modes of uncharged and charged forms of anthracycline antibiotics with CyD is available at the moment. The presence of the two substituents may cause a

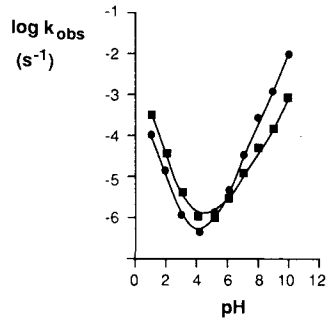

Fig. 3. Log k_{obs}-pH profile for the degradation of AD-32 in the presence of γ-CyD (3.7×10^{-2} M) (●) and HP-β-CyD (3.7×10^{-2} M) (■).

different mode of complexation and, consequently, a change in stabilization. The fact that the solubility study (Fig. 5) indicates that AD-32 undergoes complexation, in contrast with doxorubicin (Bekers et al., 1988), with α- and β-CyD is a further confirmation for the difference in complexation mode between AD-32-CyD on the one hand and doxorubicin-CyD on the other.

Influence of pH

The influence of the pH on the degradation rate of AD-32 has been investigated for γ-CyD and HP-β-CyD in the pH range 1–10. These two CyD were chosen, since they show the greatest effect on the solubility of AD-32 (see Solubility study). As can be seen from Fig. 3, in acid AD-32 is more stable in γ-CyD than in HP-β-CyD, whereas in alkaline medium the reverse effect is observed.

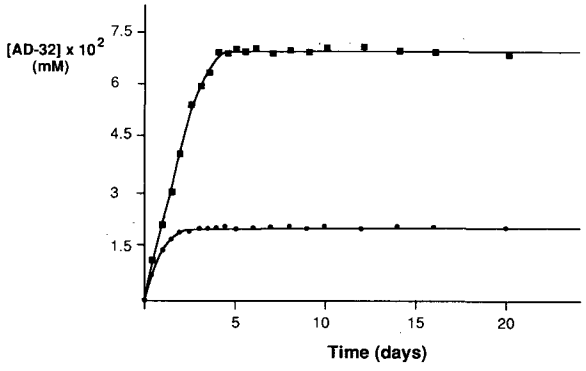

Fig. 4. Solubility rate of AD-32 in the presence of γ-CyD (●) and HP-β-CyD (■).

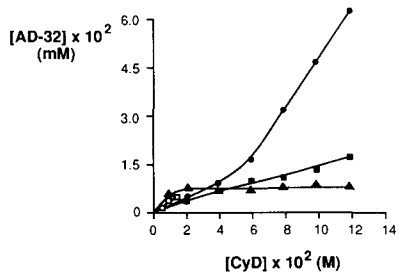

Fig. 5. Solubility isotherms of AD-32 in the presence of various CyD; (▲) = α-CyD; (□) = β-CyD; (●) = HP-β-CyD; (■) = γ-CyD.

The pH profile (Fig. 3) also shows, in accordance with earlier results (Bekers et al., 1989), that the pH of maximum stability for AD-32 is around 4. The solubility study has therefore been performed at pH 4.

Solubility study

Fig. 4 shows, with γ-CyD and HP-β-CyD as examples, that the maximum solubility of AD-32 in the presence of CyD was reached within 5 days. The same solubility rate of AD-32 occurs for the other CyD. For this reason the samples for the phase-solubility study were withdrawn 5 days after the start of an experiment. The phase-solubility diagrams obtained for AD-32 are presented in Fig. 5. It is obvious that in every case the solubility of the drug apparently increases with increasing CyD concentration. According to the definitions provided by Higuchi and Connors (1965) all curves indicate the formation of soluble inclusion complexes of the A-type.

A plot of the γ-CyD concentrations vs the quantity of dissoluted AD-32 is linear, so an A_L-type solubility diagram is obtained, indicating that a 1 : 1 complex is formed. α-CyD, on the contrary, leads to an A_N diagram. The isotherm obtained with β-CyD is, because of its low aqueous solubility (Uekama and Otagiri, 1987), not interpretable. Complexation of AD-32 with HP-β-CyD gives an A_P-type response. This may indicate that at higher CyD concentrations complexation between more than one HP-β-CyD molecule and one guest molecule occurs.

Fig. 5 also indicates that HP-β-CyD has the largest effect on the solubility characteristics of AD-32. Whether this observation will be of any importance in optimizing AD-32 formulations for pharmaceutical purposes will be a matter of further research.

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