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Kinetics of the acid-catalyzed hydrolysis of doxorubicin

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Summary

The kinetics of hydrolysis of doxorubicin were studied in 0.01–0.5 M hydrochloric acid solutions (pH 0.4–2.1). The rate of hydrolysis exhibited a first-order dependency on the doxorubicin concentration and on the hydrogen ion concentration, the specific hydrogen ion catalytic rate constant being 1.02 $M^{-1} \cdot h^{-1}$ at 37°C. An activation energy of 92.0 $kJ \cdot mol^{-1}$ was determined. The acid-catalyzed hydrolysis was discussed in relation to liquid chromatographic procedures involving acidic mobile phases and with respect to gastric degradation of the drug. It was estimated that in the gastric pH range 1–2 and with a gastric emptying half-time of 50 min about 86–98% of an oral dose would leave the stomach in intact form.

Introduction

Doxorubicin (adriamycin) (I), an anthracycline antibiotic widely used in cancer chemotherapy (Arcamone, 1981), consists of the tetracyclic quinoid aglycone doxorubicinone (II) and the amino sugar, daunosamine (III), linked together through a glycosidic bond. Like other glycosides, doxorubicin is susceptible to undergo hydrolytic degradation in acidic solution, with formation of the aglycone and the amino sugar (Scheme I) (Arcamone et al., 1969; Watson and Chan, 1978). However, no information appears to be available about the kinetics of degradation under acidic conditions, the only stability studies reported being concerned with photolytic degradation (Tavoloni et al., 1980; Williams and Tritton, 1981) and the stability in various infusion fluids (Poochikian et al., 1981).

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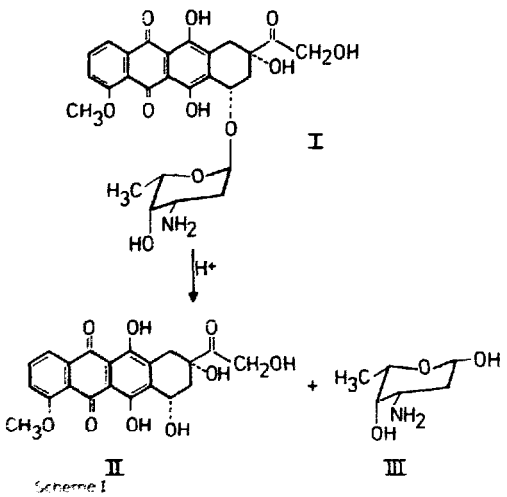

Scheme I

The purpose of this study was to provide information about the kinetics of hydrolysis of doxorubicin in acidic solutions. Knowledge about the stability at such conditions is desirable from an analytical point of view since the use of acidic mobile phases (pH 2) for liquid chromatography of doxorubicin and the related daunorubicin has been questioned because of potential degradation during the chromatographic process (Haneke et al., 1981). Furthermore, knowledge about the acid-stability may be useful to assess the extent of possible degradation of the drug in the stomach after oral administration. Thus, it has been reported that doxorubicin and the related daunorubicin are inactive when given by the oral route because of splitting of the glycosidic bond in the gastrointestinal tract (Bachur, 1976; Pratt and Ruddon, 1979). Both of these aspects are considered in this report on the basis of the kinetic data obtained.

Materials and methods

Materials

Samples of doxorubicin hydrochloride and doxorubicinone were kindly provided by FarmItalia, Milan, Italy. Buffer substances and all other chemicals and solvents used were of reagent grade. A constant ionic strength of 0.2 was maintained for each buffer solution by addition of an appropriate amount of potassium chloride.

Apparatus

The high-performance liquid chromatograph used was composed of a Waters pump 6000 A, a Waters U6K universal injector and a Schoeffel fluorescence detector FS-970. A column (15 cm × 4.0 mm i.d.) packed with LiChrosorb RP-8 (5 μm particles) (E. Merck, Darmstadt) was used.

Analytical method

Doxorubicin and doxorubicinone were determined by an HPLC method. In this method the reversed-phase RP-8 column was eluted isocratically at ambient temper-

ature with an acetonitrile–0.01 M phosphoric acid (35 : 65 v/v) mixture with pH adjusted to 2.0. The flow rate was 1.5 $ml \cdot min^{-1}$ and the column effluent was monitored spectrofluorimetrically with excitation wavelength at 470 nm and emission wavelength at 550 nm. The contents of the products in the sample injected (100 μl) into the column were determined by comparing the peak heights with those of external standards chromatographed under similar conditions.

Kinetic n easurements

All kinetic measurements were carried out in standardized hydrochloric acid solutions and at a constant temperature. The reaction solutions were kept in a thermostatically controlled water bath ($\pm 0.3°C$) in screw-capped tubes protected from light and the reactions were initiated by dissolving a weighed quantity of doxorubicin hydrochloride in the pre-heated buffer solution to give an initial concentration of about 1.2 $\mu g \cdot ml^{-1}$. The reaction progress was followed by the HPLC method described above. Pseudo-first-order rate constants were calculated from the slopes of linear plots of the logarithm of residual doxorubicin against time.

Results and discussion

In acidic aqueous solutions doxorubicin was found to hydrolyze with formation of doxorubicinone and the amino sugar daunosamine (Scheme 1) as evidenced by HPLC. In this method a peak with the same retention time as an authentic sample of doxorubicinone was observed to form simultaneously with the disappearance of the peak due to doxorubicin (Fig. 1). In 0.01–0.5 M hydrochloric acid solutions (pH 0.4–2.1) doxorubicinone was found to be formed in quantitative yields (> 95%).

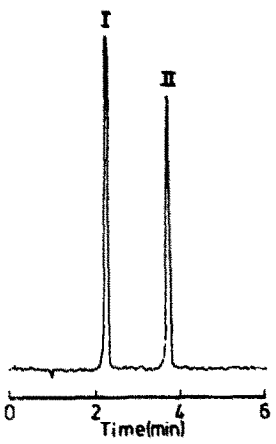

Fig. 1. A chromatogram of a partially degraded solution of doxorubicin (I) in 0.1 M hydrochloride acid showing the formation of doxorubicinone (II).

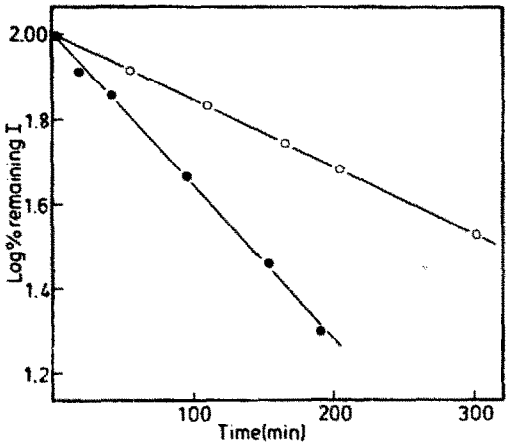

Fig. 2. First-order plots for the hydrolysis of doxorubicin (I) in 0.1 M (○) and 0.5 M (●) hydrochloric acid at 37°C.

At constant pH and temperature the hydrolysis displayed strict first-order kinetics over several half-lives. Some typical first-order plots for hydrolysis of doxorubicin are shown in Fig. 2. In the hydrogen ion concentration range investigated (0.01–0.5 M), the pseudo-first-order rate constants (k_{obs}) were found to be directly proportional to the hydrogen ion concentration as demonstrated by the rate data listed in Table 1. Thus, the kinetic results obtained can be described in terms of Eqn. 1 or Eqn. 2:

$$\text{rate} = k_H[H^+][\text{doxorubicin}] \quad (1)$$

$$k_{obs} = k_H[H^+] \quad (2)$$

where k_H is a second-order rate constant for the apparently specific acid-catalyzed hydrolysis. At 37°C and $\mu = 0.2$ k_H was calculated to be 1.02 $M^{-1} \cdot h^{-1}$. Under the conditions studied the doxorubicin is present entirely as a protonated species, the pK_a of the amino group being 7.2 (Eksborg, 1978).

The ionic strength was found to be without any significant influence on the rate of hydrolysis at values up to 0.5.

The effect of temperature on the hydrolysis rate was determined in 0.5 M hydrochloric acid solutions over the range 22–50°C. From the slope and intercept of an Arrhenius plot of the data an activation energy of 92.0 $kJ \cdot mol^{-1}$ was calculated.

In order to assess the possible significance of acid-catalyzed hydrolysis of doxorubicin for the stability of the drug in the stomach after oral administration important factors to be considered include the gastric acidity and the gastric emptying rate. These parameters vary widely among and within individuals and are influenced by numerous factors such as the emotional state and food ingestion (Cooke, 1975; Mayersohn, 1979). Normally, gastric pH is within the range 1–2 and a gastric emptying half-time of about 50 min can be taken as a reasonable mean value (Griffith et al., 1968; Heading et al., 1973; Digenis et al., 1977). Using the

TABLE 1

RATE DATA FOR THE HYDROLYSIS OF DOXORUBICIN IN DILUTED HYDROCHLORIC ACID SOLUTIONS ($\mu = 0.2$) AT 37°C

$[H^+]$ (M)	pH	k_{obs} (h^{-1})	k_H ($M^{-1} \cdot h^{-1}$)
0.01	2.13	0.0108	1.08
0.03	1.65	0.0309	1.03
0.05	1.43	0.0504	1.01
0.10	1.13	0.0995	1.00
0.50 *	0.43	0.502	1.00
		mean	1.02

* $\mu = 0.5$

relationship, valid at 37°C and $\mu = 0.2$ (Harned and Hamer, 1933):

$$\log[H^+] = 0.13\text{-pH} \tag{3}$$

and the value of k_H of 1.02 $M^{-1} \cdot h^{-1}$, the pseudo-first-order rate constants of hydrolysis (k_h) at pH 1 and 2 were calculated to be 0.14 and 0.014 h^{-1}, respectively (37°C). Assuming the gastric emptying process to be associated with a first-order rate constant (k_{ge}) of 0.83 h^{-1} (derived from a $t_{1/2}$ value of 50 min), the percentage amount of doxorubicin surviving acid-catalyzed degradation in the stomach can be calculated using the following equation for two parallel first-order processes:

$$\% \text{ undegraded doxorubicin} = \frac{k_{ge}}{k_{ge} + k_h} \times 100 \tag{4}$$

The figures derived in this way are 86% for pH 1 and 98% for pH 2. Thus, acid-catalyzed hydrolytic degradation in the stomach of orally administered doxorubicin appears to be of minor importance. The reported inactivation of doxorubicin in the gastrointestinal tract after oral administration (Bachur, 1976; Pratt and Ruddon, 1979) may thus be ascribed primarily to other processes, e.g. to enzymic hydrolysis by glycosidases. It should be added, however, that patients undergoing anticancer chemotherapy may have longer gastric emptying half-times than normally. If, for example, the emptying half-time is 4 h and the gastric pH is 1 only 55% of an ingested dose of doxorubicin can be calculated to leave the stomach in intact form.

The rate data presented may also be useful to assess the potential detrimental effect of a pH 2 mobile phase in HPLC of doxorubicin and related anthracycline antibiotics as claimed by Haneke et al. (1981). At 22°C and pH 2 (pure aqueous solution) the time needed for hydrolyzing doxorubicin to an extent of 1% can be calculated to be about 80 min. Thus, the utilization of a mobile phase with a pH of 2 appears not to be unacceptable in regard to hydrolytic degradation of the drug during the chromatographic process. This was also confirmed with the presently used mobile phase system where an extent of degradation of less than at least 1% was noted.

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