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Factors affecting the stability of ceftriaxone sodium in solution on storage

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Summary

The influence of storage temperature (37, −20, −40 and −70°C), solvent (water, 0.9% NaCl, 5, 10 and 20% dextrose) and antibiotic concentration (10, 25, 50 and 100 μg/ml) on ceftriaxone disodium bioactivity was studied by means of a quantitative bacteriological agar gel diffusion assay. The loss of bioactivity, at 37°C, occurred in two phases, rapid degradation within the first 24 h, followed by a second phase of steadily decreasing bioactivity. At freezing temperatures, the degradation of ceftriaxone followed a monoexponential first-order process. Admixtures can be stored for 3 months at −70°C, 2 months at −40°C, except 5% dextrose (t_{90} = 51 days), and 1 month at −20°C without significant loss of antibiotic bioactivity. Ceftriaxone disodium was more stable in water solution, followed by 0.9% NaCl and 5, 10 and 20% dextrose solutions. The stability of ceftriaxone decreased with increasing concentration. Drawing several samples in the first 24 h was proved to be important to determine the shelf-life (t_{90}) when it is less than 24 h.

Introduction

Ceftriaxone disodium, a semisynthetic third-generation cephalosporin antibiotic, has been shown to be effective for the treatment of a variety of serious bacterial infections. What distinguishes ceftriaxone from the other newer cephalosporins is its unusually long plasma half-life, which is 4–10-times longer than that of other cephalosporins (Neu et al., 1981; Stoeckel, 1981).

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The stability of drugs depends on several factors such as concentration of antibiotic, vehicle, pH, dielectric constant, density, ionic strength, time and storage temperature, among others (Arias and Vila, 1981).

A number of published studies have reported that certain antibiotics in clinical use may be frozen for extended periods of time without serious loss of activity (Dinel et al., 1977; Kleinberg et al., 1980; Stiles, 1981; Holmes et al., 1982; Nicolai et al., 1985). The demonstration that some of the most commonly used antibiotics have excellent stability in the frozen state for quite long periods is, therefore, of importance and some hospitals have already made beneficial use of this

finding. The technique of freezing would permit the advance preparation of antibiotic admixtures, under aseptic conditions, ensuring antibiotic stability and microbial integrity. This process may also save technician time and decrease the amount of drug wastage caused by expired compounded drug. In addition, in laboratories a stock solution is usually prepared and refrigerated (4°C) or frozen to be used in successive assays (Ausman et al., 1980; Quentin et al., 1990; Raoult et al., 1991). Samples from pharmacokinetics and stability studies of drugs are stored at freezing temperatures ranging from −20 to −70°C until the assay is performed without specifying the duration of this storage (Rodriguez-Barbero et al., 1984; Dajani and Pokowski, 1990; Fuchs et al., 1991).

The objective of this study was to determine the influence of medium, temperature, and concentration of dextrose and antibiotic on degradation kinetics of ceftriaxone disodium. Additionally, the equation best fitting the experimental data was determined.

Materials and Methods

Materials

Ceftriaxone disodium powder, batch 012037, with a potency of 820 μg as free acid/mg was provided by Roche S.A.

Solutions of 0.9% sodium chloride, 5, 10 and 20% dextrose, were prepared with the same sterile water to give pH 6.0–6.2.

The influence of antibiotic concentration was studied in 0.1 M phosphate buffer pH 7.4 with constant ionic strength of 0.5 M adjusted with sodium chloride. All chemicals and reagents were of analytical grade.

The test microorganism was *Bacillus subtilis* ATCC 6633 spores. Antibiotic media 1 and 2 from Difco and assay plates (10 cm) were used. The zone-reader was a vernier caliper (Somet Inox).

The density of solutions was measured in a pycnometer and the dielectric constant determined indirectly, in a DMO1 WTW dipolometer. Solutions were tested for pH at each assay time using a 290 MK 2 pH Meter (Pye Unicam).

Methods

Sterile ceftriaxone disodium was diluted aseptically in sterile 0.9% NaCl, 5, 10 and 20% dextrose, and water at a concentration of 50 μg/ml and in 0.1 M phosphate buffer pH 7.4 at four concentrations (10, 25, 50 and 100 μg/ml). After pH values and remnant concentration were determined, 1-ml aliquots of the solutions were placed in triplicate in sterile glass 2-ml vials and stored at −20, −40, and −70°C for 3 months and at 37°C for 2 weeks. At appropriate intervals, samples were removed and assayed after being thawed for 2 h at room temperature (when conserved at freezing temperatures) or after several minutes when stored at 37°C. The specimens were discarded after sampling.

The percentage of ceftriaxone remaining at each interval was determined by comparing concentrations at these times with the ceftriaxone concentration measured just after the initial dilution.

Bacteriological agar gel diffusion assay

The concentration of the antibiotic was determined in triplicate by a disc-plate assay procedure described in detail elsewhere (Esteban et al., 1990) using *B. subtilis* as the assay microorganism. Five working standard dilutions containing 10, 7, 5, 3 and 2 μg/ml of antibiotic were prepared in phosphate buffer solution (pH 6.0, 0.1 M) to construct the standard curve derived by linear regression from the zones of inhibition of standard solutions. Only curves with correlation coefficients (r) greater than 0.996 were used. The potency of the test solution was determined by interpolation of the standard curve.

Mathematical methods

Monoexponential ($\log C = [K/2.303] \cdot t + \log C_0$) and biexponential ($C = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$) ($C$, antibiotic concentration; A and B, constants; K, degradation constant; α, degradation constant for the rapid phase; β, degradation constant for the slow phase; t, time) equations of first-order degradation kinetics were employed to

fit the experimental data using the simplex iterative method of the MULTI 1 programme. The best fit was selected using Akaike's information criterion (AIC) (Yamaoka et al., 1978). The shelf-life (t_{90}) and half-life (t_{50}) were calculated from the equation. When the experimental data were adjusted to a biexponential equation the shelf-life (t_{90}) and half-life (t_{50}) were calculated using the iterative Newton's method (Bowman and Morgall, 1983).

Statistical method

A two-factor analysis of variance test and Scheffe test for paired comparison were employed to define significant differences in percentages of mean antibiotic test concentrations determined under protocol conditions. The a priori level of significance was $p < 0.05$.

TABLE 1

Degradation rate kinetic (K) and shelf-life (t_{90}) of ceftriaxone disodium in water, 0.9% NaCl and 5% dextrose solutions, at freezing temperatures

Solution	Temperature (°C)	K (days^{-1})	t_{90} (days)
Water	−20	−0.00297	35
	−40	−0.00153	68
	−70	ND	> 3 months
0.9% NaCl	−20	−0.00299	34
	−40	−0.00176	60
	−70	ND	> 3 months
5% dextrose	−20	−0.00314	33
	−40	−0.00206	51
	−70	ND	> 3 months

ND, no degradation in the studied period of time; K, degradation rate constant.

Results and Discussion

pH determinations

No significant increase in pH value was observed in any of the solutions studied during storage at freezing temperatures and at 37°C. Our results extend those obtained by both Walker and Kedzierewicz in the study of stability of ceftriaxone in dextrose and saline solutions (Walker and Dranitsaris, 1987; Kedzierewicz et al., 1989).

Influence of temperature

An increase in stability was achieved at low temperatures, in agreement with Arrhenius theory.

For practical purposes, the stability of an antibiotic is normally considered to be satisfactory if it maintains at least 90% of its activity under specified conditions. This convention is almost universal in the literature on the subject and is adopted here.

The degradation of ceftriaxone disodium in water, 0.9% NaCl and 5% dextrose at −20, −40 and −70°C followed a monoexponential first-order process. The degradation rate kinetics (K) and the shelf-life (t_{90}) are listed in Table 1.

Storage for 90 days at −70°C did not reduce the mean antibiotic activity below 90% of initial concentration. When stored at −40°C the remaining bioactivity was 85.33, 84.69 and 82.46% in water, 0.9% NaCl and 5% dextrose, respectively. At −20°C it was 78.45% in water, 75.56% in 0.9% NaCl, and 73.41% in 5% dextrose. Differences in degradation of antibiotic between these solutions and temperatures were significant ($p < 0.05$).

A frozen state reaction theory as proposed by Pincock and Kiovsky (1966) could be considered to explain the differences in rate and extent of degradation at these temperatures. A frozen solution will consist of both liquid and solid phases in equilibrium below the freezing point but above the eutectic temperature of the system. Under these conditions, a frozen solution cannot be considered only solid. Temperatures below the eutectic point of the solution appear to ensure antibiotic stability. For ceftriaxone disodium the eutectic temperature appears to be below −40°C. It was seen that the antibiotic in water, 0.9% NaCl and 5% dextrose solutions at −40°C was stable for over 50 days, suggesting that the eutectic point had been reached, but as they were still stable when stored at −70°C for 90 days, it could indicate that the eutectic point for each of these admixtures presumably is not as low as −70°C but below −40°C.

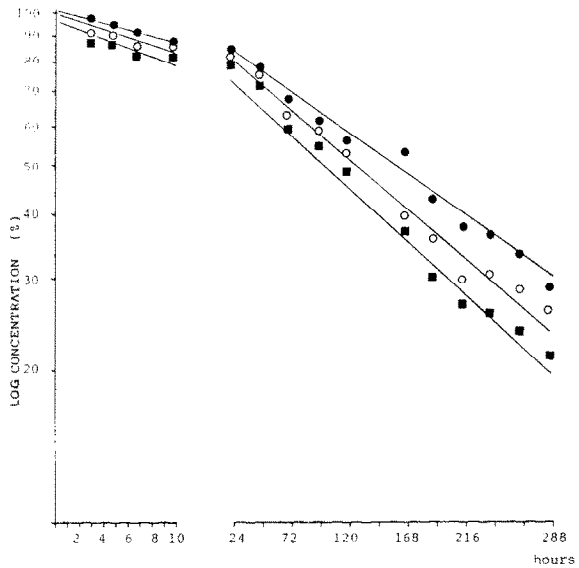

Fig. 1. Apparent first-order plots of the degradation of ceftriaxone disodium in water (●), 0.9% NaCl (○) and 5% dextrose (■) at 37°C.

The loss of approx. 10% of initial ceftriaxone concentration in 5% dextrose solution in our study conflicts with the results of Smith (1983). This discrepancy may be related to the use of different ceftriaxone concentrations because when the antibiotic concentration increases, the stability of ceftriaxone decreases.

The degradation of ceftriaxone disodium in water, 0.9% NaCl and dextrose stored at 37°C followed a biexponential first-order process (Fig. 1). The degradation occurred in two phases, with a more rapid degradation within the first 24 h. When a monoexponential equation was used to obtain t_{90} values, discrepancies between actual data and those generated by log-linear regression analysis were found, such differences being referred to a 'background' by Walker and Dranitsaris (1987). With the biexponential equation no background is found. As rapid degradation occurred in the first 24 h, it is important to draw several samples during this time in order to detect rapid degradation of antibiotics. The degradation constant for the rapid phase (α) and slow phase (β), the shelf-life (t_{90}) and the half-life (t_{50}) of these solutions are listed in Table 2.

TABLE 2

Degradation constants for the rapid (α) and slow (β) phase, shelf-life (t_{90}) and half-life (t_{50}) of ceftriaxone disodium in water, 0.9% NaCl and 5% dextrose solutions, at 37°C

Solution	Degradation constant (h^{-1})		t_{90} (h)	t_{50} (h)
	Phase α	Phase β		
Water	0.1930	0.0038	10.19	162.07
0.9% NaCl	0.1941	0.0095	7.50	131.85
5% dextrose	0.6196	0.0052	3.69	115.03

Influence of the medium and dextrose concentration

Ceftriaxone disodium was found to be less stable in dextrose solutions than in 0.9% NaCl and water (Tables 1 and 2). This cannot be attributed to the differences in pH, but to the catalytic effect of the dextrose on the hydrolysis of the antibiotic, as occurs in other β-lactam antibiotics (Bundgaard and Larsen, 1970; Chatterji et al., 1975).

The influence of dextrose concentration was studied at 37°C in 5, 10 and 20% dextrose solutions. In this case, the degradation kinetics occurred in two phases and when dextrose concentration increased, the t_{90} decreased. Table 3 shows the degradation constant for the rapid phase (α) and slow phase (β), the shelf-life (t_{90}) and the half-life (t_{50}) in these solutions.

The two-factor analysis of variance test and the Scheffe test for paired comparison showed significant differences between all dextrose solutions ($p < 0.05$).

TABLE 3

Degradation constants for the rapid (α) and slow (β) phase, shelf-life (t_{90}) and half-life (t_{50}) of ceftriaxone disodium in 5%, 10% and 20% dextrose solutions, at 37°C

Solution	Degradation constant (h^{-1})		t_{90} (h)	t_{50} (h)
	Phase α	Phase β		
5% dextrose	0.6196	0.0052	3.69	115.03
10% dextrose	0.4591	0.0057	2.46	97.84
20% dextrose	0.3728	0.0064	1.92	77.82

TABLE 4

Dielectric constants and densities of the solutions

Solution	Dielectric constant	Density (g/ml)
Water	80.3702	0.9967
0.9% NaCl	32.4280	1.0046
5% dextrose	30.2015	1.0147
10% dextrose	29.2235	1.0327
20% dextrose	27.4249	1.0629

The differences in stability between ceftriaxone solutions could be explained by the Debye-Hückel theory (Carstensen, 1970) as shown by the equation:

$$\log K = B + 2.825 \times 10^6 \left[\frac{\delta}{\epsilon^3 \cdot T^3}\right]^{\frac{1}{2}} \cdot Z_a \cdot Z_b \left[\frac{\sqrt{\mu}}{1+\sqrt{\mu}}\right]$$

where K is the degradation rate kinetic constant, B the rate constant at zero ionic strength, δ the density, ϵ the dielectric constant, T the absolute temperature, $Z_a Z_b$ the electric charges of the reactants conducting to the activate complex and μ the ionic strength.

Table 4 shows the dielectric constant and density of the solutions studied. Dextrose solutions have a greater density and a smaller dielectric constant than 0.9% NaCl and water solutions. It also explains the greater degradation rate kinetics found in dextrose solutions.

Influence of antibiotic concentration

Antibiotic concentration was studied at the pH of maximum stability and constant ionic strength to avoid interactions between pH and ionic strength as shown by Rodriguez-Barbero et al. (1984) and Martinez-Pacheco et al. (1987). This pH was also selected, since it is the pH of human plasma.

The logarithm of percentages of ceftriaxone disodium remaining at time intervals in phosphate buffer at 37°C is illustrated in Fig. 2. The change is antibiotic concentration followed a

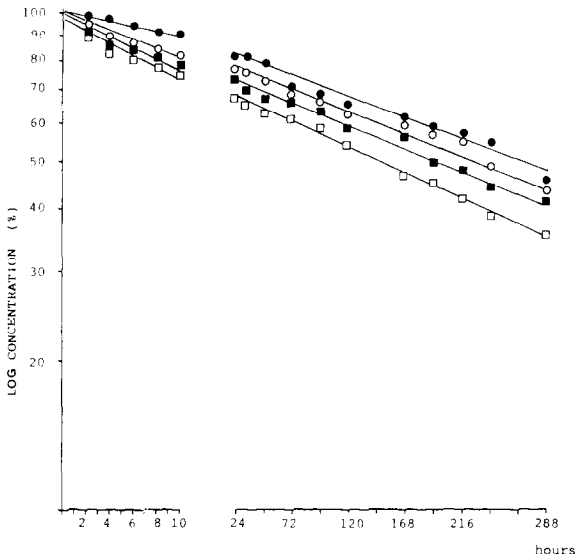

Fig. 2. Apparent first-order plots of ceftriaxone disodium degradation at several antibiotic concentrations: 10 μg/ml (●), 25 μg/ml (○), 50 μg/ml (■) and 100 μg/ml (□), in 0.1 M phosphate buffer pH 7.4, at 37°C.

first-order degradation process and the data were fitted to a biexponential equation. The degradation constant for the rapid phase (α) and slow phase (β), the shelf-life (t_{90}) and half-life (t_{50}) in these solutions are listed in Table 5.

Fig. 3 shows the logarithm of t_{90} plotted vs the logarithm of the ceftriaxone concentration. The log t_{90} was linearly dependent on the logarithm of ceftriaxone concentration, with a correlation coefficient of 0.920 ($p < 0.05$).

The two-factor analysis of variance test showed significant differences between the concentra-

TABLE 5

Degradation constants for the rapid (α) and slow (β) phase, shelf-life (t_{90}) and half-life (t_{50}) of ceftriaxone disodium in different antibiotic concentration solutions, at 37°C

Ceftriaxone concentration (μg/ml)	Degradation constant (h^{-1})		t_{90} (h)	t_{50} (h)
	Phase α	Phase β		
10	0.0630	0.0015	12.76	294.80
25	0.1307	0.0018	4.22	265.27
50	0.1429	0.0021	3.99	204.34
100	0.1447	0.0023	3.01	115.03

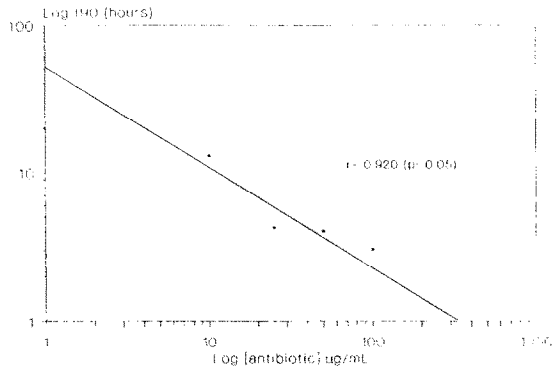

Fig. 3. Apparent first-order shelf-life (t_{90}) of ceftriaxone disodium vs antibiotic concentration.

tions of antibiotic studied, as did the Scheffe test for paired comparison ($p < 0.05$).

Kedzierewicz also observed that the stability of ceftriaxone in intravenous solutions varies according to the antibiotic concentration (10–50 mg/ml) for a given temperature and solvent (Kedzierewicz et al., 1989).

Regarding the results shown in Table 5, one must take into account that when samples from biological fluids are used for pharmacokinetic or antibiotic stability studies it is important to refrigerate or freeze them as soon as possible, to avoid loss of activity. If high concentrations are expected or used, storage time before determination should be short. Demotes-Mainard et al. (1988) and Esteban et al. (1990) observed that plasma samples containing 40 and 155 μg/ml of ceftriaxone could be kept at −20°C for 1 month in agreement with our results, and those containing 10 μg/ml could be stored at 4°C or at ambient temperature for 2 days.

Conclusions

Ceftriaxone disodium in water, 0.9% NaCl and 5% dextrose solution can be stored for 3 months at −70°C; solutions in water and 0.9% NaCl for 2 months at −40°C and 5% dextrose solution for 50 days at the same temperature; furthermore, the three solutions can be stored for 1 month at −20°C without loss of bioactivity. The eutectic point for each of these admixtures presumably is not as low as −70°C but is below −40°C.

The stability of ceftriaxone disodium varies according to the antibiotic concentration and solvent. It has been proved that ceftriaxone disodium degradation fits best to a biexponential equation, which is of considerable practical importance especially when the shelf-life (t_{90}) must be established.

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