Pharmaceutical Properties of Loracarbef: The Remarkable Solution Stability of an Oral 1-Carba-1-dethiacephalosporin Antibiotic[1]

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Received June 24, 1991; accepted September 3, 1991

Loracarbef is an oral 1-carba-1-dethiacephalosporin antibiotic structurally related to cefaclor. Like many β-lactam antibiotics, loracarbef exists in several hydrated crystalline forms. The pH–solubility profile curve for loracarbef monohydrate is U-shaped, resembling those for other zwitterionic cephalosporins. Loracarbef was found to be much more stable in solution than cefaclor. For example, in pH 7.4 phosphate buffer, loracarbef was unexpectedly found to be 130–150 times more stable than cefaclor and 10–12 times more stable than cephalexin, depending on the phosphate concentration. The pH–stability profile is U-shaped, similar to that of other zwitterionic cephalosporins, and shows maximum stability at the isoelectric point. At any given pH, loracarbef is more stable in solution than any other therapeutically useful cephalosporin. Acetate, borate, citrate, and especially phosphate buffers have catalytic effects on the rate of loracarbef hydrolysis.

KEY WORDS: loracarbef; 1-carba-1-dethiacephalosporin; 1-carbacephem; β-lactam; solution stability.

INTRODUCTION

The "1-carba-1-dethiacephalosporins" first prepared by Christensen are a class of β-lactam antibiotics in which the 1-S atom of the cephalosporin nucleus has been replaced by a CH_2 (methylene) moiety (1). Since 1974 several laboratories have reported on the synthesis and antimicrobial evaluation of 1-carbacephems (2,3). In general, the 1-carbacephems possess similar antimicrobial activity and decreased chemical reactivity compared to the corresponding cephalosporin (3–6). The human pharmacology and pharmacokinetics of loracarbef (Fig. 1), the 1-carbacephem analogue of cefaclor, have recently been reported (7). We now report the pharmaceutical properties of loracarbef, 7-[D-(aminophenylacetyl)amino]-3-chloro-8-oxo-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid and its remarkable solution stability compared to cefaclor and cephalexin.

MATERIALS AND METHODS

Loracarbef monohydrate and dihydrate were supplied by the Chemical Process Research and Development group of Lilly Research Laboratories. Cephalexin monohydrate and cefaclor monohydrate were obtained from production lots (Eli Lilly and Company). For clarity and consistency when discussing the various hydrates of loracarbef, cephalexin, and cefaclor, we specify loracarbef monohydrate, cephalexin monohydrate, and cefaclor monohydrate when we are referring to the monohydrate crystal forms. In reality the USAN name for each compound refers to the monohydrate crystal form. All other chemicals were of reagent grade. X-ray powder diffraction patterns were acquired with a Nicolet 12V automated diffractometer. The diffractometer was equipped with a Cu-Kα radiation source and graphite diffracted beam monochromator. The X-ray diffraction patterns were collected using step scan conditions of 5 sec/step, 0.05° 2θ/step, from 4 to 35° 2θ.

Analytical Procedures

HPLC. Compounds were quantitated using a reverse-phase 4.4 × 250 mm Zorbax ODS column, a 1 ml/min flow rate, and a UV detector set at 254 nm. The mobile phases (v:v) were MeCN/0.025 *M* $NH_4H_2PO_4$ (10:90) for loracarbef and cefaclor and MeOH/0.1 *M* NH_4OAc (35:65) for cephalexin. Peak areas were measured with a Hewlett-Packard 3390A integrator and concentrations were calculated from standard curves.

Colorimetric Assay. The phenylglycyl side-chain primary amino group was quantitated by using a modified trinitrobenzenesulfonic acid assay (8). A 400-μl sample of the reaction solution was added to 2.0 ml of a pH 7.6, 0.2 *M* phosphate buffer solution. To this colorless solution was added 2.0 ml of a 0.2% (w/v) aqueous solution of 2,4,6-trinitrobenzenesulfonic acid, and the mixture was allowed to react (in the dark) at room temperature. After 30 min, the absorbance of the resulting yellow-orange solution was measured at 420 nm against a blank consisting of 400 μl of reaction solution without loracarbef, 2.0 ml of phosphate buffer, and 2.0 ml of trinitrobenzenesulfonic acid solution treated similarly. Concentrations of loracarbef free amino group were calculated from standard curves.

Vapor Pressure Isotherms

Crystalline powders were initially assayed for water content by Karl Fischer and crystal form by X-ray powder diffraction pattern. Accurately weighed samples were suspended in jars containing saturated solutions (with excess solute) of various salts known to give constant humidities in closed environments. The samples were assayed for changes in weight with time (typically equilibration occurred in 1 to 3 days) and the new water content was calculated from the weight change and initial water content. When significant changes in weight were observed the samples were also assayed for changes in crystal form by X-ray powder diffraction pattern and for reversibility of the weight change by transferring a sample to a different jar(s) of appropriate relative humidity.

Aqueous Solubilities

The aqueous solubilities were determined by suspending the crystalline compounds in water at 37°C, rapidly ad-

[1] Presented, in part, at the Western Regional Meeting of the AAPS, Reno, Nevada, February 27–March 1, 1989.

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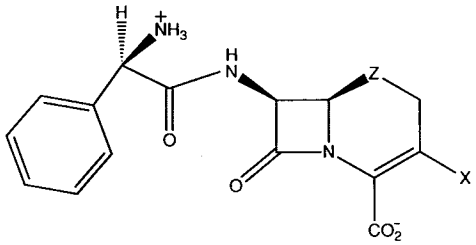

Loracarbef: Z=CH_2, X=Cl

Cefaclor: Z=S, X=Cl

Cephalexin: Z=S, X=CH_3

Fig. 1. Structures for loracarbef, cefaclor, and cephalexin.

justing the pH appropriately with either NaOH or HCl, and equilibrating at constant pH on a pH-stat for 1 hr. Equilibration time was limited to 1 hr because of stability concerns for cefaclor and because loracarbef equilibrium solubility, at least at the isoelectric point, occurs in significantly less than 1 hr. Samples of the suspension were removed, filtered, appropriately diluted, and assayed by HPLC.

Kinetic Methods

The hydrolysis rates were determined by following loss of parent β-lactam by HPLC. For the very slow rates, $T_{1/2} >$ 1500 hr, the reaction was followed through ≈10% completion; for all other rates the reaction was followed through one to two half-lives. All solutions (pH stat and buffer) were adjusted to μ = 0.5 with KCl. The solutions were maintained at 35°C except for the Arrhenius studies. An accurately weighed sample of compound was dissolved in 0.5 *M* KCl for pH stat experiments or the appropriate buffer solution preheated to the desired temperature to give initial β-lactam concentrations of 7×10^{-4} to 1×10^{-3} *M*. Samples were taken at appropriate time points and assayed immediately. At pH 10 and 11, a pH stat was used to maintain constant pH by addition of NaOH. The reaction vessel was overlaid with argon. At pH 1 and 2, 0.1 and 0.01 *N* HCl were used, respectively. At other pH's buffer solutions were used: acetate at pH 4 and 5, citrate at pH 4.6, phosphate at pH 6 and 7.4, and borate at pH 8 and 9. The pH was rechecked at the last time point and no significant changes in pH were observed. For the pH–stability profile, rate constants for these pHs were calculated by extrapolating rate constants from several buffer concentrations to infinite dilution. Pseudo-first-order kinetics were observed for all conditions. Where standard errors are given, the rate constants were determined three times in separate experiments. All other rate constants are an average of two experiments with the exception of the rate constants at pH 4.6 (citrate buffer), which were each determined from a single experiment.

RESULTS AND DISCUSSION

Crystal Pseudopolymorphism

Crystal pseudopolymorphism has been observed for several zwitterionic β-lactam antibiotics and has played an important part in understanding the processing and formulation of ampicillin (9), cephaloglycin (10), cephalexin (10–12), and cephadroxil (13). Loracarbef exists in a monohydrate and a dihydrate crystal form (Fig. 2), depending upon the method of crystallization. The monohydrate form is obtained by crystallization from an aqueous solution at a temperature of approximately 50°C (14). The needle-shaped crystals exhibit a unique X-ray powder pattern (Fig. 3) and contain approximately 1 mol of water when air equilibrated at 10 to 70% relative humidity (RH). Below 10% RH the water is lost and the X-ray powder pattern decreases in intensity; above approximately 80% RH nonstoichometric water is absorbed with no change in the X-ray powder pattern. If the dehydrated material is exposed to ≥10% RH a mole of water is reabsorbed and the original monohydrate X-ray powder pattern returns.

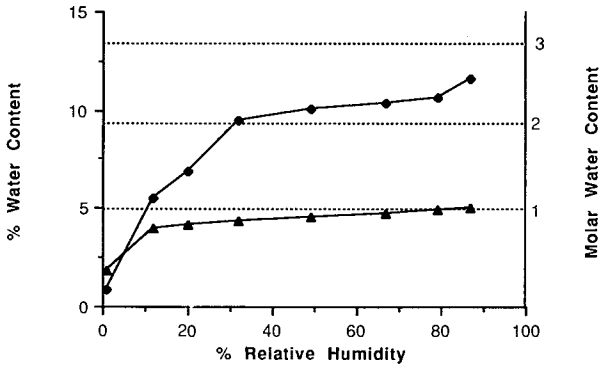

Fig. 2. Water sorption/desorption isotherms for crystalline loracarbef monohydrate (▲) and dihydrate (♦).

The dihydrate form is obtained by crystallization from an aqueous solution at low temperature, 5 to 10°C (15). The plate-shaped crystals contain approximately 2 mol of water when equilibrated at 35 to 90% RH. Below 35% RH the water is lost and the X-ray powder pattern decreases in intensity. If the dehydrated dihydrate crystals are exposed to ≥35% RH, water is reabsorbed (to approximately the 2 mol level) and the dihydrate X-ray powder pattern of original intensity returns. Crystallizations performed at room temperature give mixtures of the monohydrate and dihydrate crystal forms. In contrast to cephalexin (10) and cefaclor, no interconversion from loracarbef monohydrate to dihydrate crystals or dihydrate to monohydrate crystals has been observed during the formulation and reconstitution of pediatric

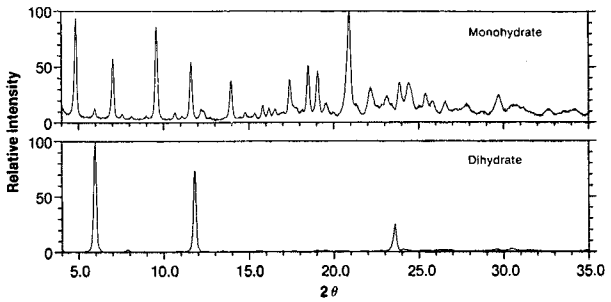

Fig. 3. X-ray diffraction powder patterns of crystalline loracarbef monohydrate and dihydrate.

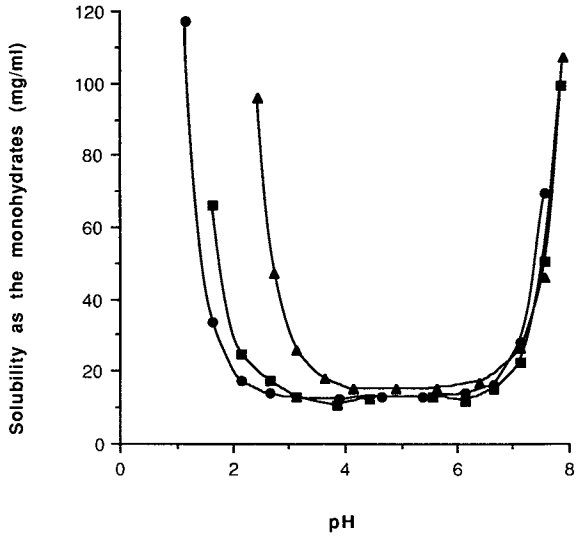

Fig. 4. The effect of pH on the aqueous solubility of loracarbef monohydrate (■), cefaclor monohydrate (●), and cephalexin monohydrate (▲) suspended in water at 37°C.

forms of this antibiotic (either as a solid–solid transformation where water vapor molecules are incorporated into the crystal lattice or by a solution-mediated process where crystals are slurried in water at ≤25°C).

Aqueous Solubility

The loracarbef monohydrate pH–solubility profile at 37°C (Fig. 4) is U-shaped, resembling that for the zwitterionic cephalosporins, cefaclor monohydrate and cephalexin monohydrate. However, in contrast to both cefaclor and cephalexin monohydrates, which immediately convert to the dihydrate crystal forms when suspended in water, loracarbef maintains its monohydrate crystal form. Minimum solubility, 10.1 mg/ml, is observed near pH 4.6, the isoelectric point, and solubility increases rapidly as the carboxyl group (pK_a, 2.0) is protonated or the amino group (pK_a, 7.3) is deprotonated. The minimum solubilities for cefaclor and cephalexin (calculated as the monohydrates) are 10.6 and 13 mg/ml, respectively. The solubility value of 13 mg/ml for cephalexin is somewhat lower than the published value of 17.2 mg/ml in 0.5 *M* KCl (16).

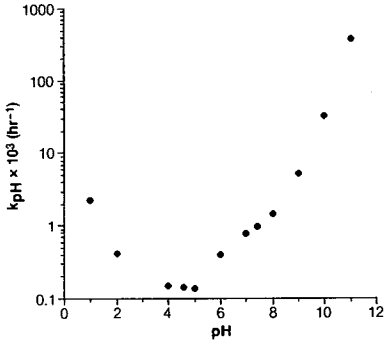

Fig. 5. The log k_{pH}–pH profile for loracarbef hydrolysis at 35°C, μ = 0.5.

Solution Stability

The solution stability of β-lactam (and γ-lactam) antibi-

Table I. Effects of pH and Buffer Concentration on the Pseudo-First-Order Hydrolysis Rates of Loracarbef (1), Cefaclor (2), and Cephalexin (3), at 35°C and μ = 0.5 (HPLC Assay)

Compound	pH (buffer)	$k_{obs} \times 10^3$ hr^{-1} 0.05 *M*	0.1 *M*	0.15 *M*	0.2 *M*	$k_{pH} \times 10^3$ hr^{-1}
1	1 0.1 *N* HCl					2.23 ± 0.28
	2 0.01 *N* HCl					0.420 ± 0.020
	4 (acetate)	0.171	0.195	0.209	0.235	0.151[a]
	4.6 (citrate)	0.206	0.285	0.347	0.406	0.144[a]
	5 (acetate)	0.204	0.255	0.301	0.388	0.138[a]
	6 (phosphate)	0.819	1.35	1.67	2.21	0.389[a]
	6 (phosphate)	(0.742)[b]	(1.15)[b]	(1.38)[b]	(1.90)[b]	(0.367)[a,b]
	7.4 (phosphate)	2.10	3.44	4.51	5.70	0.970[a]
	7.4 (phosphate)	(1.72)[b]	(3.15)[b]	(3.79)[b]	(4.94)[b]	(0.825)[a,b]
	8 (borate)	1.57	1.85	1.96	2.09	1.45[a]
	9 (borate)	5.59	6.17	6.39	6.72	5.32[a]
	10 pH stat					32.4 ± 2.7
	10 pH stat					(9.46)[b]
	11 pH stat					372 ± 22
	11 pH stat					(63.9)[b]
2	7.4 (phosphate)	269	479 ± 4	663	847	85.0[a]
	7.4 pH stat					50.7 ± 4.6
	10 pH stat					944 ± 74
3	7.4 (phosphate)	25.1	35.8 ± 3	48.4	66.0	10.0[a]
	10 pH stat					68.5 ± 3.4

[a] Values calculated by extrapolating rate constants at various buffer concentrations to infinite dilution.
[b] Rate constants are for loss of free amino group as measured by the colorimetric assay.

Table II. Effects of pH and Buffer Concentration on the Apparent Arrhenius Activation Energies for Loracarbef Hydrolysis

pH	Phosphate (*M*)	$k_{obs} \times 10^3$ hr^{-1} 35°C	40°C	45°C	50°C	55°C	60°C	65°C	E_a[a] (kcal/mol)
10		32.4	84.4	183	340		1270	2510	29.3
7.4	0.05	2.10	3.82			16.7		45.8	21.0
7.4	0.1	3.44	5.97			26.5		65.7	20.3
7.4	0.15	4.51	7.96			35.3		86.7	20.3
7.4	0.2	5.70	10.0			41.6		102	19.8

[a] The apparent E_a at pH 7.4 and pH 10 are similar to those obtained for other β-lactam antibiotics (16, 26–29.)

otics has received considerable attention in the literature from two general aspects: first, from a structure–activity point of view, where the chemical reactivity of the lactam ring with hydroxide ion (usually pH 9 to 11) has been correlated with microbiological activity (17–23); second, and more commonly, from a pharmaceutical point of view, where solution stability may influence dosage form design, assay methodology, and an understanding of the pharmacokinetics of the antibiotic (24–30). For cefaclor and cephalexin there is a correlation between β-lactam reactivity (at pH 10, 35°C, $\mu = 0.5$, $k = 0.944$ hr^{-1} and $k = 0.0685$ hr^{-1}, respectively) and microbiological activity. The 14-fold more chemically reactive cefaclor is two to four times more microbiologically active than cephalexin against most organisms and 16-fold for *Haemophilis influenzae*. However, when loracarbef is compared with cefaclor and cephalexin, the correlation breaks down. The microbiological activity of loracarbef is almost identical to that of cefaclor, but loracarbef is chemically less reactive (at pH 10, 35°C, $\mu = 0.5$, $k = 0.0324$ hr^{-1}) than both cefaclor (29-fold) and cephalexin (2-fold). A similar pattern of almost-identical microbiological activity with decreased chemical reactivity has been reported for several pairs of 1-carba-1-dethiacephalosporins and their corresponding cephalosporin analogues (4). At pH 7.4 (Table I) the decrease in chemical reactivity of the β-lactam ring of loracarbef translates into even greater differences in relative stability compared to cefaclor and cephalexin. Loracarbef is 130–150 times more stable than cefaclor and 10–12 times more stable than cephalexin at pH 7.4, depending on the phosphate buffer concentration.

The differences in relative reactivity of the β-lactam rings at pH 10 and solution stability at pH 7.4 (phosphate buffer) for loracarbef, cefaclor, and cephalexin may be due to differences in reaction mechanism. At pH 10 the predominant reaction mechanism is attack of hydroxide ion on the β-lactam ring, whereas at pH 7.4 intramolecular nucleophilic amine attack on the β-lactam (8,22,25,29) [catalyzed by phosphate ion (8,25)] may compete with attack of hydroxide ion. For loracarbef at pH 6.0 and 7.4 the rate of loss of free $-NH_2$, as measured by Bundgaard's colorimetric assay (Table I), is similar to the rate of loss (by HPLC) of parent loracarbef, whereas at pH 10 and 11 the loss of free $-NH_2$ is significantly slower than the loss of parent loracarbef. However, we must point out that neither our laboratory nor that of Dr. L. Lorenz, Dr. S. Baertschi, and Mr. M. Skibic (Analytical Development of Lilly Research Laboratories) have been able to isolate any 2,5-piperazine-dione products to support this hypothesis. Neither borate, citrate, nor acetate ion (Table I) has as large a catalytic effect on the stability of loracarbef as phosphate ion. The loracarbef pH–stability profile (Fig. 5) is similar in shape to the pH–stability profiles of most cephalosporins, especially phenylglycine-substituted cephalosporins (8,16,26–29), but loracarbef is the most stable cephalosporin at any pH. The Arrhenius activation energies, (Table II) 29.3 kcal/mol at pH 10 and approximately 20.4 kcal/mol at pH 7.4, support the hypothesis of different decomposition mechanisms.

CONCLUSIONS

Loracarbef is similar to cefaclor in structure, solubility, and microbiological activity, but it is significantly more stable in solution than cefaclor or any other therapeutically useful cephalosporin. The excellent microbiological activity of loracarbef, coupled with its remarkable solution stability, suggests a clinical future for loracarbef and other 1-carbacephem antibiotics.

ACKNOWLEDGMENTS

We wish to thank Mr. Gregory Stephenson for obtaining and interpreting the X-ray powder patterns. We also acknowledge Dr. Arvind Thakkar for sharing data on the time course for loracarbef equilibrium solubility at the isoelectric point.

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