Stability of the New Anticancer Platinum Analogue 1,2-Diaminomethyl-Cyclobutane-Platinum(II)-Lactate (Lobaplatin; D19466) in Intravenous Solutions

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The chemical stability of the new anticancer platinum analogue 1,2-diaminomethyl-cyclobutane-platinum(II)-lactate (D19466) in infusion media was studied in an accelerated stability testing experiment with a selective HPLC-UV method. Variables were time, temperature, light, concentration, and infusion mixture. Mean reaction rate constants of decomposition were, respectively, $0.9555 * 10^{-2}$, $2.127 * 10^{-2}$, and $4.221 * 10^{-2}$ hr^{-1} at 37, 56, and 66°C at a concentration of 200 mg/L in normal saline. From the Arrhenius equation, shelf lives (5% loss) at 4, 22, 37, and 121°C were, respectively, calculated to be 41.6, 13.2, 5.7, and 0.15 hr. Mean reaction rate constant in 5% dextrose was $3.106 * 10^{-2}$ hr^{-1} (200 mg/L; 56°C) and differed from that in normal saline ($P < 0.005$). Mean reaction rate constant in Ringer lactate was $2.084 * 10^{-2}$ hr^{-1} (200 mg/L; 56°C) ($P > 0.05$). There was no influence of normal daylight on the rate of decomposition. It is recommended to prepare D19466 infusions in normal saline. Chemical stability is then maximal 12 hr at room temperature or 24 hr at 4°C. No protection against normal daylight is required. Sterilization by heat is not possible.

KEY WORDS: platinum; high-performance liquid chromatography (HPLC); cisplatin analogue; D19466; lobaplatin; 1,2-diaminomethyl-cyclobutane-platinum(II)-lactate; drug stability; shelf life.

INTRODUCTION

Cisplatin has become an active component in the treatment of a number of malignancies. Side effects such as neurotoxicity and nephrotoxicity limits its applicability (1). This and the development of drug resistance have stimulated the search for new platinum compounds (2,3). Now platinum derivatives of a third generation [e.g., lobaplatin (D19466), zeniplatin (CL286,558), enloplatin (CL287,110)] are developed in the hope to obtain less toxicity and increased activity (4–6).

New platinum analogues are selected also on the basis of higher water solubility and drug stability. The stability of cisplatin in intravenous solutions is well documented (7–10), whereas data on the stability of second generation derivatives are scarce (11). As the exchange of the negatively charged ligands was shown to be related to plasma protein binding of the drug (10,12), differences in drug stability may also have influence on the pharmacokinetic properties of the drug under study. Further, aquated species which are formed from cisplatin in solutions without chloride, have been shown to be more nephrotoxic but less effective than the parent compound (13).

1,2-Diaminomethyl-cyclobutane-platinum(II)-lactate (D19466) is a recently developed third-generation platinum analogue, active against a number of experimental tumors, including a cisplatin-resistant human tumor cell line (4).

This study was performed to characterize the stability of the new drug in infusion media with a selective high-performance liquid chromatographic (HPLC) method.

MATERIALS AND METHODS

Drugs and Chemicals

D19466 (Fig. 1) was supplied by Asta Pharma (Frankfurt, Germany). Chemicals for chromatography were of analytical grade and obtained commercially. Acetonitrile of chromatographic quality and ultrapure water (Milli-Q Water Purification System, Millipore Waters, Etten-Leur, The Netherlands) were used for analysis.

The 0.9% sodium chloride, 5% dextrose, and Ringer lactate USP XXII infusion mixtures were prepared and packed in glass bottles with a bromobutyl rubber (FM157 Helvoet Pharma BV, Alken, Belgium) at our Department of Hospital Pharmacy.

Assay

D19466 was determined by a newly developed HPLC method. A Supelcosil LC-1 5-μm column (250 × 4.6 mm) (Art. No. 5-8296; Supelchem BV, Leusden, The Netherlands), a Model WISP 710A automatic injector system (Waters, Etten-Leur, The Netherlands), a Model L6000 pump (Merck, Amsterdam, The Netherlands) (flow rate, 1.0 ml/min), a Model SP 4290 integrator (Spectra Physics, Eindhoven, The Netherlands), and an UV-VIS detector (Spectroflow 757, Separations Analytical Instruments BV, Hendrik Ido Ambacht, The Netherlands) set at 230 nm (0.01 AUFS at an attenuation of 4) were used. The mobile phase consisted of a 0.1 *M* phosphate buffer (pH 7.0) and acetonitrile (90:10%, v/v).

D19466, 9.88 mg, dissolved in 1000 ml water was used as standard. Twenty microliters of sample or freshly thawed standard was injected into the HPLC. Concentrations were calculated by peak area ratio of sample over standard. Samples were cooled (4°C) in the automated injector system during analysis.

Infusion Preparation and Sampling

Intravenous infusion mixtures were prepared with concentrations of 40, 200, or 400 mg D19466 per liter, commensurate with those used in the clinic. Preequilibrated 0.9%

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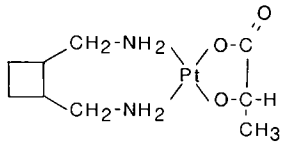

Fig. 1. Chemical structure of D19466.

sodium chloride, 5% dextrose, and USP XXII Ringer lactate were used as diluents. Decomposition at 37, 56, and 66°C in an oven was followed by taking 100-µl samples at appropriate intervals. Samples were diluted with water by the use of a Model 1500 Syva automatic pipettor-diluter (Sunnyvale, CA) to 10 mg D19466 per liter.

Drug content was also determined at 4 and 22°C (200 mg/L in normal saline) in order to check the results of the accelerated stability testing experiment. Samples (n = 3) were taken and measured just after preparation and again after 96 hr. All diluted samples were injected into the HPLC immediately.

Light dependence was studied by placing three infusions (200 mg/L in normal saline) at room temperature by daylight, by UV light (254 nm), and in the dark. Samples were taken and measured just after preparation and again after 90 hr.

Data Analysis

The accelerated stability study is based upon the equation of Arrhenius, which relates the rate of the decompensation reaction to temperature (14):

$$\ln k = \ln A - (E/R * T)$$

where A is the frequency factor (no dimension), k is the reaction rate constant (hr^{-1}), E is the activation energy (J $*mol^{-1}$), R is the gas constant (8.314 J · mol^{-1} · K^{-1}), and T is the absolute temperature (K).

The reaction rate constants (k) at 37, 56, and 66°C were calculated from the slopes of the logarithmic presentation of the concentration (% of initial value)-versus-time curves. The linear relationship of $\ln k$ versus the reciprocal of absolute temperature offers the opportunity to calculate A and E. These values were obtained by regression analysis by the method of least squares.

The order of the reaction was assessed by the graphical method and by calculation of the coefficients of correlation for zero-, first-, and second-order reaction kinetics.

Reaction rate constants at several temperatures were calculated from the Arrhenius equation by substitution. Shelf lives were calculated from the equation

$$c/c_o = e^{-k * t}$$

where c/c_o is the actual concentration/initial concentration (0.95 or 0.90), k is the reaction rate constant (from the Arrhenius equation), and t is the time (hours to 5 or 10% loss). The stability in normal saline was studied most comprehensively as the manufacturer recommends reconstitution in this diluent. Drug contents at 4 and 22°C after 96 hr were determined to check the calculated shelf lives from the Arrhenius equation.

Statistical Analysis

Mean reaction rate constants were analyzed for significance by the two-sided Student's t test. P values <0.05 are considered significant.

RESULTS AND DISCUSSION

Chromatography

Platinum-containing substances are frequently assayed by flameless atomic absorption spectrophotometry (F-AAS). This method cannot be used for stability studies, as the parent compound cannot be distinguished from its platinum-containing degradation products. In recent years several selective methods have been developed to measure platinum-containing anticancer drugs.

The HPLC method used here offers the opportunity to measure D19466 selectively. Chromatograms of a fresh and a decomposed infusion are depicted in Fig. 2. D19466 has a retention time of 5.9 min. Decomposition was forced by heat and led to a strong reduction of the peak height at 5.9 min and the appearance of an alternative peak at 8.5 min. Calibration curves were prepared in fourfold (0, 2.47, 4.94, 7.41, and 9.88 mg/L) and found to be linear (mean coefficient of correlation = 0.9991; n = 4; injection volume = 10 µl). The limit of detection (defined as three times the signal-to-noise ratio) appears to be 0.1 mg/L (injection volume = 40 µl; AUFS = 0.005; Att = 4). The coefficient of variation appears to be 2.6% at 2.5 mg/L (n = 8; injection volume = 10 µl).

Stability

The percentage D19466 of its initial concentration-versus-time curves at 37, 56, and 66°C in normal saline are presented in Fig. 3. Stability of infusion mixtures in normal

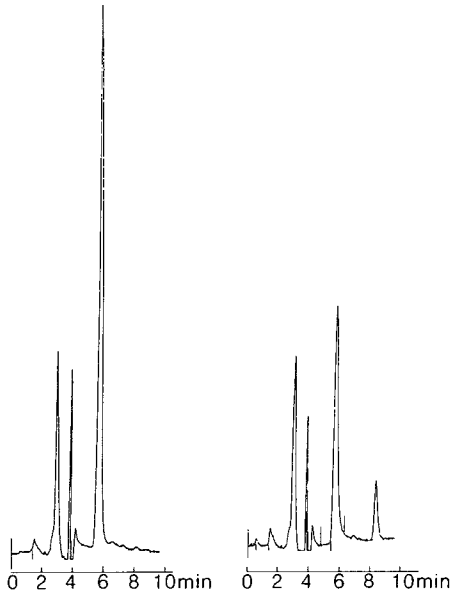

Fig. 2. Chromatograms of a fresh and decomposed sample of D19466 in normal saline: R_t (5.9 min) = D19466, and R_t (8.5 min) = decomposition product of D19466.

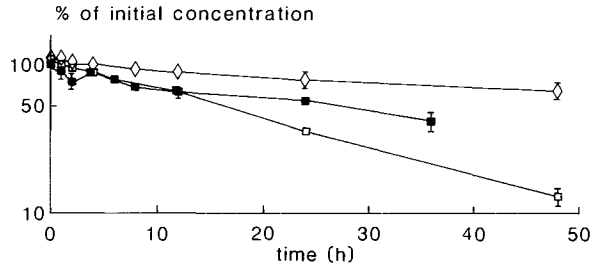

Fig. 3. Drug stability of D19466 (200 mg/L) in 0.9% sodium chloride at 37°C (—◇—), 56°C (—■—), and 66°C (—□—) [log % versus time (hr); mean ± SD; n = 3]. The SD fall within the symbol, unless stated otherwise.

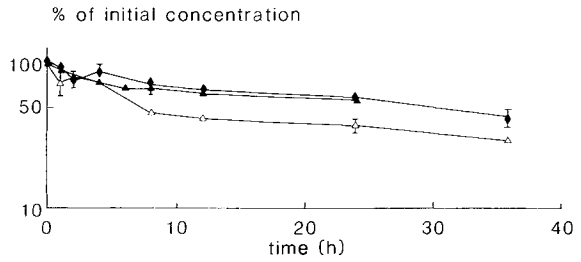

Fig. 5. Drug stability of D19466 (200 mg/L) in 5% dextrose (—△—), USP XXII Ringer lactate (—▲—), and 0.9% sodium chloride (—◆—) at 56°C [log % versus time (hr); mean ± SD; n = 3]. The SD falls within the symbol, unless stated otherwise.

saline at 40, 200, and 400 mg/L is shown in Fig. 4, and stability of infusions in 5% dextrose and in Ringer lactate in Fig. 5. A (pseudo) first-order reaction resulted in the best fit. The calculated reaction constants of the various infusions are depicted in Table I. From logarithmic regression analysis of k versus $1/T$, the Arrhenius equation can be completed into (r = −0.99):

$$\ln k = 12.1430 - 5219.1/T$$

Calculated reaction rate constants and shelf lives (90 and 95% of initial concentration) at several temperatures are given in Table II. In practice, shelf lives of D19466 at 200 mg/L normal saline of 24 and 12 hr at 4 and 22°C, respectively, seems most suitable. Because of its instability at high temperatures, autoclaving or other thermical treatments for sterilization of D19466 should be discouraged. Upon standing at 4 and 22°C (200 mg/L in normal saline) a mean concentration of 88.11 ± 0.86% and 84.30 ± 5.85%, respectively, was found 96 hr after preparation (n = 3). From the accelerated stability experiment a drug content of 88.8 and 68.8%, respectively, could be calculated at these temperatures.

Dissolved in 5% dextrose the drug is more unstable in comparison to normal saline ($P < 0.005$). The explanation for this might be given by the lower pH of dextrose infusion. The pH of the diluents were 4.8, 5.6, and 6.8, respectively, for dextrose, sodium chloride, and Ringer lactate. A pH-dependent decomposition was also shown for JM-40 (15). With cisplatin, chloride concentration is an important factor in decomposition (7,8). The decomposition of cisplatin is described by exchange of one of the two chloride groups for water, reaching an equilibrium dependent upon the initial cisplatin concentration. The second chloride group can also be displaced, but in the overall decomposition reaction this is of minor importance (8,10). The reaction is reversible; infusions containing cisplatin are therefore formulated with sodium chloride as stabilizing agent.

D19466 has a leaving group of lactate and not of chloride ions. The stability of D19466 in Ringer lactate was therefore studied to determine if this had a stabilizing effect. In Ringer lactate there are different possible ligands in solution: lactate (28 mM), chloride (110 mM), and water. The stability of D19466 in Ringer lactate was not significantly different from normal saline.

When dissolved at an initial concentration of 40 mg/L normal saline the drug appears to be more unstable than at 200 mg/L ($P < 0.0025$), whereas at a concentration of 400 mg/L the reaction rate constant is decreased, however, significance is not reached ($P > 0.05$).

Sensitivity for light was tested at room temperature; after 90 hr the concentration of D19466 was 88.67 ± 3.24, 85.32 ± 2.64, and 81.25 ± 4.44% in dark, daylight, and UV light, respectively [$P < 0.05$ (UV light) and NS (daylight) compared to dark].

Comparing to data from the literature it appears that D19466 in normal saline is more stable than unstabilized cisplatin solutions (water or 5% dextrose) (15). When stabilities of cisplatin and D19466 in normal saline at room temperature are compared, it appears that cisplatin is more stable. In normal saline and at room temperature, D19466 was found to be approximately as stable as carboplatin (11) but much more stable than JM-40 (15). However the dilution of carboplatin in normal saline is not recommended because of the possible conversion to cisplatin (11). In Ringer lactate, cisplatin was also found to be stable (25°C) even when exposed

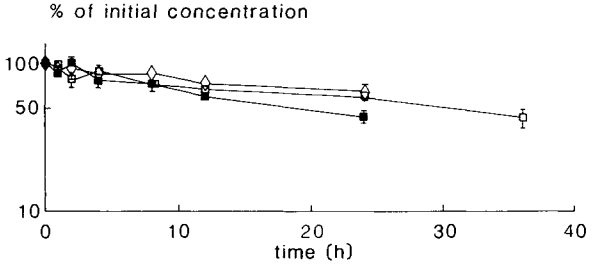

Fig. 4. Drug stability of D19466 in 0.9% sodium chloride at 56°C and a concentration of 40 mg/L (—■—), 200 mg/L (—□—), and 400 mg/L (—◇—) [log % versus time (hr); mean ± SD; n = 3]. The SD falls within the symbol, unless stated otherwise.

Table I. Reaction Rate Constants (Mean ± SD; n = 3) of D19466 in Infusion Media

Temp. (°C)	Conc. (mg/L)	Infusion	Reaction rate constant (hr^{-1})
37	200	0.9% NaCl	$0.956 * 10^{-2} \pm 0.0719 * 10^{-2}$
56	200	0.9% NaCl	$2.127 * 10^{-2} \pm 0.3136 * 10^{-2}$
56	40	0.9% NaCl	$3.432 * 10^{-2} \pm 0.2697 * 10^{-2}$
56	400	0.9% NaCl	$1.720 * 10^{-2} \pm 0.1044 * 10^{-2}$
56	200	Ringer lactate	$2.084 * 10^{-2} \pm 0.1555 * 10^{-2}$
56	200	5% dextrose	$3.106 * 10^{-2} \pm 0.2191 * 10^{-2}$
66	200	0.9% NaCl	$4.221 * 10^{-2} \pm 0.0956 * 10^{-2}$

Table II. Calculated Shelf Lives (5 or 10% Loss) of D19466 at a Concentration of 200 mg/L of Normal Saline at Several Temperatures

Temp.		Reaction rate constant	Shelf life (hr)	
°C	K	(hr^{-1})	5% loss	10% loss
4	277	$0.123 * 10^{-2}$	41.6	85.5
22	295	$0.389 * 10^{-2}$	13.2	27.1
37	310	$0.916 * 10^{-2}$	5.6	11.5
41	314	$1.135 * 10^{-2}$	4.5	9.3
100	373	0.157	0.33	0.67
120	393	0.321	0.16	0.33
121	394	0.332	0.15	0.32

to light. Such stability almost certainly is the result of the high chloride ion concentration in Ringer lactate USP (7).

CONCLUSION

It is recommended to dispense D19466 in normal saline infusions at concentrations of 40–400 mg/L. Shelf lives are 12 hr at room temperature or 24 hr at 4°C. Protection from normal daylight is not necessary. The use of 5% dextrose for reconstitution and low drug concentrations should be avoided, whereas USP XXII Ringer lactate can be used as an alternative diluent, but no stabilizing effect of lactate could be observed. Solutions cannot be sterilized by thermical treatment.

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