Precipitation of the Renin Inhibitor Ditekiren[1] Upon iv Infusion; *in Vitro* Studies and Their Relationship to *in Vivo* Precipitation in the Cynomolgus Monkey

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Ditekiren is a pseudo-octapeptide being developed as an inhibitor of human renin. Preclinical drug safety studies with this drug involved continuous iv infusions through indwelling catheters in the right internal jugular vein of the cynomolgus monkey for up to 30 days. The following physicochemical properties of ditekiren make it susceptible to intravascular precipitation immediately following iv infusion: (1) the water solubility of ditekiren is high at acidic pH where the drug is formulated (pH 4) but low at physiologic pH, and (2) the water solubility of ditekiren decreases by roughly 50% from room temperature (25°C) to physiologic temperature (37°C). Studies of 28- and 30-day infusion durations revealed intravascular precipitation in monkeys using drug solutions and rates of infusion that were expected to be precipitation-free, based on the solubility of ditekiren and assumptions about blood flow in the monkey right internal jugular vein. Therefore, an *in vitro* apparatus was used to study the relationship among the drug concentration in the infusate, the rate of infusion, and the occurrence of precipitation in a fluid stream of phosphate-buffered bovine serum albumin solution (a facsimile of plasma). Maximum rates of infusion without precipitation were determined for a range of concentrations of drug in two separate formulations. Infusion conditions identified by the *in vitro* method as precipitation-free were then tried in a definitive 14-day monkey study. Of 24 monkeys infused with solutions of ditekiren, none showed evidence of intravascular precipitation. This study demonstrates that the *in vitro* precipitation system is useful in establishing drug concentrations and rates of injection which avoid the problem of intravascular precipitation in preclinical animal studies.

KEY WORDS: ditekiren; renin inhibitor; intravascular precipitation; plasma compatibility; peptides; formulations.

INTRODUCTION

Ditekiren is a pseudo-octapeptide which is being developed as an inhibitor of circulating human renin for the treatment of hypertension. The pharmacologic activity of ditekiren in rats and monkeys has been described in previous publications (1–3).

Preclinical drug safety studies involved intravenous (iv) infusion of cynomolgus monkeys with solutions of ditekiren. Two aspects of ditekiren water solubility raise the possibility of intravascular precipitation. First, the water solubility of ditekiren is high at acidic pH where the drug is formulated (20 mg/ml solubility at pH 4) but relatively low at physiologic pH (0.3 mg/ml at pH 7.4). Second, ditekiren water solubility decreases with increasing temperature from 0.35 mg/ml at 25°C to 0.165 mg/ml at 37°C (both at pH 7.0). Infusion of ditekiren in its acidic formulation into the bloodstream will place ditekiren in a pH and temperature environment where it is significantly less soluble.

A preliminary preclinical drug safety study in monkeys showed intravascular precipitation in all dose groups. In an attempt to investigate this observation, an *in vitro* precipitation study patterned after Yalkowsky *et al.* (4) was conducted to define injection rates and drug concentrations which would minimize the risk of intravascular precipitation. This paper describes the *in vitro* precipitation system and the results of a 14-day infusion study in monkeys which was carried out using infusion parameters predicted by the *in vitro* system as acceptable in terms of avoiding intravascular precipitation. This study demonstrates that this *in vitro* precipitation system is useful in establishing drug concentrations and rates of injection which minimize the potential of intravascular precipitation in preclinical studies.

MATERIALS AND METHODS

Materials

All compounds were used as received: ditekiren (The Upjohn Company), 5% dextrose-USP (Travenol), and bovine serum albumin (Sigma Chemical Company).

In Vitro Precipitation Apparatus

The *in vitro* precipitation apparatus was assembled as depicted in Fig. 1. The various components of the system included a Brinkman RM-6 water bath, a Rainin Instrument Company peristaltic pump, a Razel syringe pump, a Milton Roy Spectrophotometer 1001, a Perkin Elmer R100A strip chart recorder, Technicon 2.5-mm-I.D. manifold tygon tubing, and a Becton Dickinson Hypodermic needle (1.5 in., 20 G).

The water bath heated a reservoir of running buffer to 52°C. Cooling of the running buffer as it passed through the tubing caused its temperature in the flow cell to be about 37°C. This was verified using a "T" tube with a thermometer inserted in the middle port which was positioned just after the flow cell. Buffer solution consisting of 20 m*M* NaH_2PO_4, 140 m*M* NaCl, 3% bovine serum albumin, pH adjusted to 7.4 with 1 *N* NaOH, was pumped through the apparatus at a rate of 15 ml/min. The injection site consisted of a 20-G needle inserted into the tygon tubing 12 cm above the flow cell and secured using a dab of Krazy Glue. Syringes containing drug or placebo solutions were attached to this secured needle.

[1] Ditekiren was formerly designated U-71,038. Its chemical name is t-butyloxycarbonyl-L-prolyl-L-phenylalanyl-*N*(alpha)-methyl-L-histidyl-2*S*,4*S*,5*S*-(5-amino-4-hydroxy-2-isopropyl-7-methyl octanoyl)-L-isoleucyl-2-pyridinyl-methylamide.

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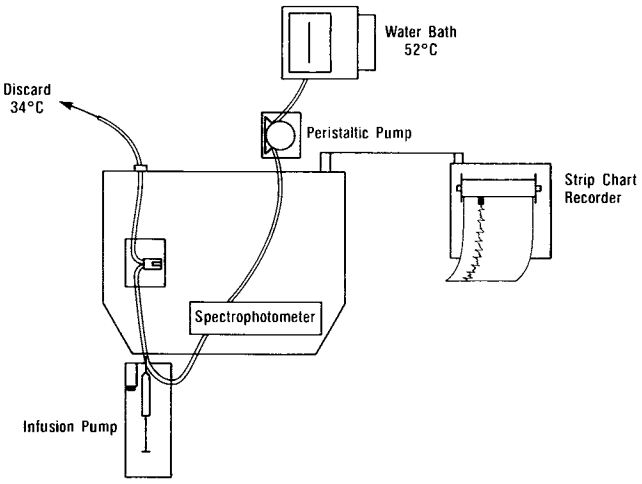

Fig. 1. Diagram of the apparatus for detecting ditekiren precipitation.

The 12-cm distance between the injection site and the flow cell was the shortest distance the equipment would allow. This distance was chosen to maximize the detection of precipitate which was most obvious immediately downstream from the injection site.

Ditekiren Solutions

Ditekiren solutions in dilute HCl were prepared by diluting a stock solution of 10 mg ditekiren/ml, 0.0192 *M* HCl, 4.45% dextrose with 5% dextrose to the desired drug concentration. Ditekiren solutions in citric acid were prepared by diluting a stock solution of 10.0 mg ditekiren/ml, 0.0323 *M* citric acid, 4.32% dextrose with 5% dextrose. Vehicle control solutions were identical to the above solutions except that they did not contain drug.

Detection of Precipitate

Precipitation of the drug from solution resulted in scatter of light at 400 nm which appeared as noise on the spectrophotometer output. Noise patterns for drug and vehicle solutions were compared at a given flow rate and any differences were attributed to drug precipitation. The differences between vehicle and drug solutions were graded as "0" (no precipitation), "+/o" (possible precipitation), "+" (definite precipitation), and "++" or "+++" (increasingly more obvious precipitation). Examples of this grading scheme are shown in Fig. 2.

In Vivo Studies

Three separate monkey studies used cynomolgus monkeys (3–6.5 kg) with surgically implanted indwelling catheters in the right internal jugular vein. The internal jugular indwelling catheter system utilized in these monkey studies is similar to that described by McNamee *et al.* (5). Intravascular precipitate was detected upon necropsy and microscopic examination of representative lung tissue. Intravascular precipitate from a preliminary study was analyzed by HPLC and found to contain significant concentrations of parent drug (data not shown).

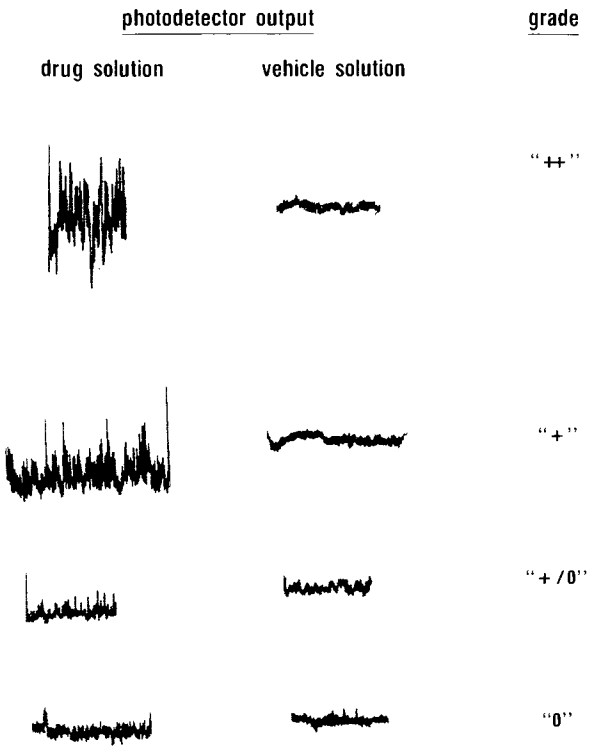

Fig. 2. Illustrations for the quantification of precipitate. The above tracings are of spectrophotometer outputs at 400 nm during the infusion of either drug solution or vehicle solution at a given infusion rate. The noisier traces associated with drug solutions are attributed to the scattering of light by drug precipitate. The grades are an attempt to quantify the amount of drug precipitate by rating the dissimilarity between the drug solutions and vehicle solutions.

RESULTS

Intravascular Precipitation in Preclinical Monkey Studies

Two monkey studies of 28- and 30-day duration were conducted using ditekiren concentrations in the infusate ranging from 1.3 to 22.4 mg/ml. Solutions were infused at either 1.3 or 2.7 ml/hr. Intravascular precipitation was detected as drug emboli in the heart and lungs. Figure 3 summarizes the occurrence of intravascular precipitation in individual animals in terms of the concentration of ditekiren infused and the rate of infusion. Data labeled I and II were obtained from each of these two studies.

The infusion parameters used in these studies were not expected to result in intravascular precipitation according to the following logic. Blood flow through a monkey right internal jugular vein was estimated to be in excess of 5 ml/min (300 ml/hr). If ditekiren is restricted to the plasma compartment which comprises about 50% of the total blood volume, then the fluid into which drug can dissolve amounts to at least 180 ml/hr. A drug solution infused at 1.3 ml/hr would undergo at least a 140-fold dilution in the bloodstream. That is, an infusate at 3 mg/ml would be diluted to 0.02 mg/ml at the injection site, assuming rapid mixing. Solutions of 10 and 20 mg ditekiren/ml would be diluted to 0.07 and 0.14 mg/ml at the injection site, respectively. All of these concentrations fall below the solubility of ditekiren (0.165 mg/ml) at physi-

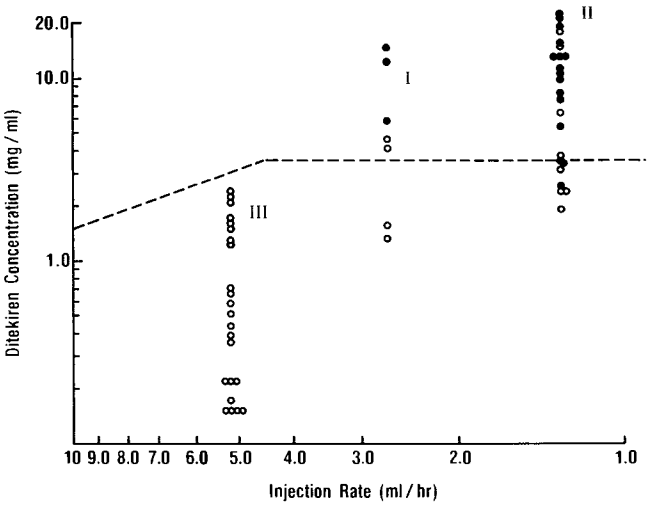

Fig. 3. Precipitation of ditekiren *in vivo;* compilation of results from cynomolgus monkeys. Each data point indicates the presence (●) or absence (○) of intravascular ditekiren precipitate in a single monkey infused with the drug solution at the rate indicated. The dashed line is the so-called "precipitation line" taken from Fig. 4. Data set I is a 28-day preliminary study, data set II is a preliminary 30-day study, and data set III is a definitive 14-day study.

ologic temperature and pH. Thus, on the basis of the above assumptions of blood flow and on the equilibrium solubility of ditekiren, we would not have expected to see intravascular precipitation. Since precipitation was seen in all dose groups, equilibrium solubility measurements are unreliable for predicting infusate concentrations and rates of infusion which avoid intravascular precipitation.

In Vitro Precipitation of Ditekiren

An *in vitro* apparatus (Fig. 1) was used to define the relationship among the drug concentration in the infusate, the rate of infusion, and the occurrence of precipitation in a fluid stream of phosphate-buffered bovine serum albumin solution flowing at 15 ml/min. Precipitation was detected using a spectrophotometer equipped with a flow-through cuvette. Precipitate was quantitated by comparison of noise patterns of drug solutions with those of their respective vehicle controls. The comparison with vehicle controls was necessary because the mixing of a clear drug solution with the slightly yellow running buffer created Schlieren lines in the flow cell. The noise resulting from these lines could not be distinguished from low-level precipitation without comparing to a vehicle control.

The precipitation data are summarized in Fig. 4. Infusion of a solution containing 10 mg ditekiren/ml in dilute HCl resulted in precipitation even at the lowest rate of infusion (0.9 ml/hr). On the other hand, solutions containing 0.5 or 0.3 mg ditekiren/ml in dilute HCl were infused at rates up to 90 ml/hr without apparent precipitation. Intermediate concentrations of 3.0 and 1.0 mg/ml in HCl began to show precipitation at 4.5 and 20.0 ml/hr, respectively. These precipitation conditions are indicated by a "precipitation line" which approximates the maximum rate of infusion for any concentration of drug that is precipitation-free (dashed line in Fig. 4). Since all solutions above 3 mg/ml showed precipitation,

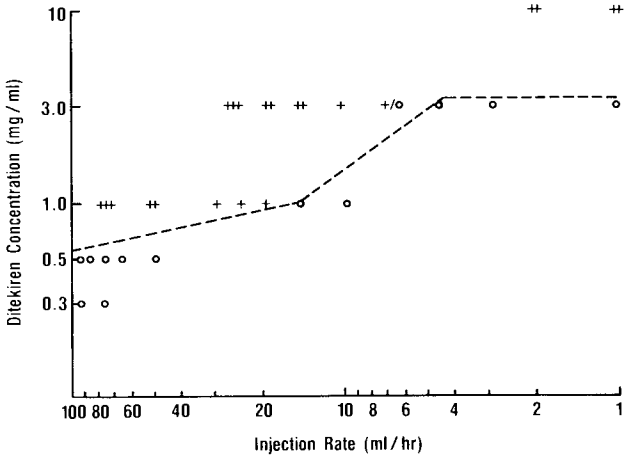

Fig. 4. Precipitation of ditekiren *in vitro;* HCl solution. Solutions were prepared as described under Materials and Methods. The amount of precipitate was subjectively assessed as described in Fig. 2.

the line was drawn horizontally from a point slightly above 3 mg/ml.

A similar study was conducted with ditekiren in a citric acid solution (Fig. 5). Precipitation occurred at slightly slower rates of infusion for concentrations of 1 and 3 mg/ml in the citric acid solutions. Thus, ditekiren shows a slightly higher propensity to precipitate from the citric acid solution.

Intravascular Precipitation of Ditekiren in a Definitive 14-Day Monkey-Study

The dashed line in Fig. 3 is the precipitation line transposed from the *in vitro* data in Fig. 4. This line was used as a guide in identifying infusion parameters which would avoid intravascular precipitation in a definitive 14-day monkey study (Group III in Fig. 3). Solutions of approximately 2.0, 0.6, and 0.2 mg ditekiren/ml were infused at 5.2 ml/hr (note that all concentrations and rates of infusion fall below the precipitation line in Fig. 3). All animals were found free of

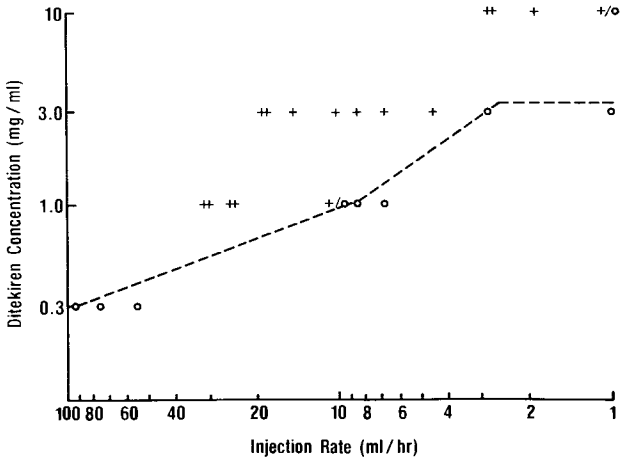

Fig. 5. Precipitation of ditekiren *in vitro;* citric acid solution. Solutions were prepared as described under Materials and Methods. The amount of precipitate was subjectively assessed as described in Fig. 2.

intravascular precipitation based upon necropsy and microscopic examination of representative lung tissue.

DISCUSSION

Intravascular precipitation in our animal studies could not be predicted correctly on the basis of equilibrium solubility measurements and assumptions about blood flow in the cannulated blood vessel. There were at least two reasons for this. First, blood flow in the cannulated right internal jugular vein of the cynomolgus monkey was probably less than the 5 ml/min used in making our initial predictions. Cannulae can actually occlude vessels in which they are implanted, thereby reducing or eliminating blood flow. Thus, the extent to which formulated drug is diluted into the bloodstream is unknown.

Second, another factor which could have hindered our ability to predict intravascular precipitation is nonequilibrium (transient) precipitation at the injection site as a result of slow mixing of infusate into the bloodstream but with rapid pH equilibration. Transient precipitation was seen in the *in vitro* study at the point of injection even under infusion conditions which diluted the drug in the infusate to well below saturated conditions. The transient precipitate readily dissolved *in vitro* upon mixing. *In vivo,* however, transient precipitate may be more persistent due to the higher viscosity of blood as well as protein and cellular factors which may adhere to the precipitate. These factors could have slowed the dissolution of transient precipitate to an extent that it became trapped in lung capillaries where it remained indefinitely.

Our data do not allow us to claim a rigorous correlation between the *in vivo* and *in vitro* results. Nevertheless, the *in vitro* experiments were quite useful in preparing for the definitive monkey study which turned out to be precipitation free. The *in vitro* experiments allowed us to compare two formulations to determine which was less prone to result in precipitation. The HCl solutions appeared slightly better in terms of preventing precipitation. The *in vitro* experiments also demonstrated that solutions above 10 mg/ml resulted in precipitation even at extremely slow infusion rates. This was consistent with our studies in monkeys which showed considerable precipitation with ditekiren concentrations of 10–20 mg/ml. Finally, the *in vitro* experiments indicated that ditekiren at 0.5 mg/ml or less could be infused at rapid rates without even transient precipitation. Thus, the finding that concentrations of approximately 0.5 mg/ml could be infused without precipitation in the definitive monkey study was anticipated.

Numerous negative consequences result from intravascular precipitation of drug in animal pharmacology and in preclinical drug safety studies. Pharmacologic and toxicologic manifestations are not expressed commensurate with the dose administered. Also, precipitated drug may create its own pathology. Intravascular precipitation can compromise drug safety studies to the point that they need to be repeated at considerable expense in time and animals. We have found the *in vitro* precipitation experiments described in this paper to be useful as a guide in establishing drug concentrations and rates of infusion which avoid intravascular precipitation in an iv infusion monkey study.

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