In Vitro Evaluation of the Plasma and Blood Compatibility of a Parenteral Formulation for Ditekiren, a Novel Renin Inhibitor Pseudopeptide

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Ditekiren (U-71038; Boc-Pro-Phe-N-MeHis-Leu-Ψ[CHOHCH3]-Val-Ile-(aminomethyl)pyridine) is a potent renin inhibitor peptide and was formulated for clinical intravenous administration in acidified dextrose. This formulation of ditekiren was evaluated in vitro with human and monkey plasma as to its potential for forming a precipitate either of drug or of plasma proteins. Analysis by centrifugation showed that no drug precipitation occurred in plasma from either species at concentrations 25 times higher than anticipated in clinical studies. Results obtained by turbidimetric measurement showed that formulated ditekiren did not cause aggregation of human platelets or flocculation of proteins at concentrations approaching the solubility limit of the drug in plasma. Ditekiren or vehicle also caused no detectable lysis of red cells at concentrations representing 10 times the maximum clinical level. Therefore, ditekiren solutions as formulated were judged completely compatible with blood and plasma upon clinical intravenous administration.

KEY WORDS: ditekiren; hemolysis; plasma compatibility; platelet aggregation; precipitation; renin inhibitor peptide.

INTRODUCTION

Ditekiren (U-71038; Fig. 1) is a highly potent inhibitor of human renin which has been demonstrated to exert prolonged hypotensive effects in animal pharmacologic models both after intravenous and oral administration (1). This compound, which is a transition-state analogue of the natural peptidic human renin substrate (2), is resistant to enzymatic hydrolysis in vivo (3) and is presently being clinically tested for the treatment of hypertension (4,5). Prior to initiating clinical studies involving the intravenous administration of this drug, a series of in vitro tests were undertaken to evaluate the compatibility of a parenteral formulation of ditekiren with plasma and erythrocytes. The potential for parenteral ditekiren to form precipitates of drug or protein when mixed with plasma, to effect the aggregation of platelets, and to induce hemolysis of red cells was evaluated for this pseudopeptide over a range of concentrations.

Materials and Methods

Materials

Ditekiren (Boc-Pro-Phe-N-MeHis-Leu-Ψ[CHOHCH3]-Val-Ile-(aminomethyl)pyridine) was synthesized at The Upjohn Company and had a chemical purity of ≥99%. [Prolyl-3H]Ditekiren (Fig. 1) was prepared at NEN Research Products (Boston, MA) by incorporating Boc-[3,4-3H]proline during the synthetic route developed by Thaisrivongs and coworkers (6). The labeled drug was purified at Upjohn to 98.3% radiochemical purity (HPLC) and had a specific activity of 35.2 mCi/mg.

A formulation of unlabeled ditekiren was prepared by dissolving 1000 mg of the drug in 89 ml of 5% dextrose solution (Injection, U.S.P.) to which had been added 1.92 ml of 1.0 M HCl. The resulting solution was diluted to 100 ml with water, giving a drug concentration of 10 mg/ml in 4.45% dextrose containing 0.0192 M HCl. This nearly isotonic solution was diluted with 5% dextrose to prepare solutions containing 5, 4, 2, 1, and 0.2 mg/ml. A placebo formulation with a buffering capacity similar to that of the 10-mg/ml ditekiren solution, but representing 0.0 mg/ml (placebo), was prepared by dissolving sodium acetate trihydrate (2.86 mg/ml) in the same vehicle. This placebo stock solution was diluted with isotonic saline or dextrose to prepare vehicle solutions having buffering capacities analogous to diluted ditekiren solutions. The pH of the 10-mg/ml ditekiren and placebo solutions was 3.3. [3H]Ditekiren solution was separately prepared by dissolving in the same dextrose/HCl vehicle at a concentration of 61.4 μg and 2.16 mCi per ml.

Parenteral phenytoin solution (phenytoin sodium injection, USP; Dilantin; Parke-Davis, Morris Plains, NJ) as a 50-mg/ml aqueous solution containing 40% propylene glycol and 10% ethanol, v/v (pH adjusted to 12 with NaOH), was obtained commercially.

Citrated fresh human plasma (American Red Cross, Lansing, MI) was pooled and pressure filtered (GF/F glass microfiber filter, 0.7 μm, Whatman Inc., Clifton, NJ), and the pH adjusted to 7.4 with 0.2 M HCl. Blood was collected from four cynomolgus monkeys, Macaca fascicularis, by venipuncture into syringes containing citrate phosphate dextrose anticoagulant solution (14 ml per 100 ml of blood) (7). Plasma was harvested by centrifugation for 30 min at 1200g. The pooled plasma was then filtered and the pH adjusted as described above.

Fresh human blood was collected from healthy volunteers by venipuncture and treated with sodium citrate (0.38%, w/v) to prevent coagulation. Platelet-rich human plasma was prepared by centrifuging fresh blood for 10 min at 200g. The resulting platelet count was found to be 2.6 × 10^9 platelets mm^-3 of plasma.

Solubility Measurements in Plasma and Buffer

To 10 ml of plasma or isotonic phosphate-buffered saline (8) in a glass culture tube was added 1.0 ml of formulated ditekiren as well as an aliquot (50 μl) of the corresponding radioactive drug in vehicle. The mixture was vigorously vortex agitated and then incubated for 30 min at 37°C.
RESULTS AND DISCUSSION

Solubility of Ditekiren in Plasma and Buffer

Ditekiren (Fig. 1) is a renin inhibitory peptide which is currently under clinical evaluation for the treatment of hypertension. Ditekiren is weakly basic and was formulated in acidified dextrose solution at approximately pH 3.3 to prepare concentrations of up to 10 mg/ml. As the pH approaches physiologic or neutral values, the solubility diminishes rapidly to about 200 µg/ml in buffer at pH 7.4 at room temperature. This compound also exhibits an inverse solubility/temperature relationship, being somewhat more soluble in aqueous solutions at lower temperatures. Because of the limited solubility of ditekiren at physiologic pH and temperature, the potential exists that drug precipitation could occur as the formulation mixes with blood at the site of infusion. In one safety study in monkeys (11), intravascular drug emboli were observed at the higher dose levels and rates of infusion. In order to assess the possibility of formulated ditekiren to intravascularly precipitate during clinical tolerance trials, the solubility in human plasma, monkey plasma, and buffer was investigated in vitro.

We first estimated the maximum local ditekiren concentration expected in human blood at the site of infusion. The average arterial blood flow (12) to the human forearm is 3.1 ml/min per 100 ml of forearm volume. Since the average forearm volume is ca. 1200 ml, this represents a total venous forearm blood flow of 37 ml/min. In turn, the flows of individual antecubital veins can be estimated as about 12 ml/min by dividing the number of major veins across the elbow into the forearm blood flow. The maximum concentration of formulated drug to be used clinically is 0.2 mg/ml, which is to be infused at a maximum rate of 1.2 ml/min (5). Thus, at the site of infusion for each 1-min interval, 0.24 mg of drug will be mixed with a total fluid volume of about 13 ml (12 ml blood + 1.2 ml vehicle), giving an estimated maximum concentration of about 18.5 µg/ml. For defining a drug concentration range to be used in compatibility studies, this approximate concentration value was rounded upward to 20 µg/ml. Based on the above reasoning, the volume ratio of vehicle added to blood per minute would be forecasted as about 1.2:12 (1:10) at the maximum infusion rate.

To define and compare the limits of drug solubility in plasma and in isotonic buffer, solutions of radiolabeled ditekiren in the dextrose/HCl vehicle were mixed at increasing concentrations with pooled fresh human and monkey (cynomolgus; Macaca fascicularis) plasma and with isotonic phosphate-buffered saline, keeping the volume ratio of formulated drug to plasma or buffer constant at 1:10. After incubation (37°C for 30 min), the mixture was agitated to resuspend any precipitates, and then an aliquot was removed for radioactivity determination. Further aliquots were then centrifuged to remove any suspended material, and the radioactivity in the supernatant was compared to that prior to centrifugation. The results (average of experiments run in duplicate) of these solubility measurements are presented in Fig. 2. The change in ditekiren concentration after centrifugation is presented as a percentage of the original, uncentrifuged concentration. Data are presented for increasing drug concentrations in human plasma, monkey plasma, and buffered saline.