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Research paper

Pharmacokinetics of human recombinant C1-esterase inhibitor and development of anti-drug antibodies in healthy dogs

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ABSTRACT

Complement-mediated intravascular hemolysis occurs in canine immune-mediated hemolytic anemia (IMHA). Complement inhibitors such as recombinant C1 esterase inhibitor (rC1-INH) might prevent this process and alter the disease course. This study aimed to characterize the pharmacokinetics of a single 500 IU IV dose of rC1-INH in 8 healthy beagle dogs, evaluate the dogs for any adverse effects of drug administration, and determine whether rC1-INH administration induces anti-drug antibody formation. Serum rC1-INH concentrations were measured using a commercial functional ELISA at baseline and at 10, 20, 40, 60, 80, 100, 120, 240, 360, 480, 600, 720, 960, and 1440 min post drug administration. Complete blood counts were conducted at baseline, 720 and 1440 min. Western blot analysis, using rC1-INH as the target antigen was used to detect anti-drug antibodies in 14-day serum samples. No adverse clinical reactions were noted following rC1-INH administration. Pharmacokinetic modelling suggested that the peak C1-INH concentration achieved is 0.21 IU/mL and that C1-INH concentration is significantly greater than baseline for 100 min following injection. A robust antibody response was detected which suggests that rC1-INH should not be re-administered after an initial course. Clinical trials of rC1-INH in dogs with intravascular IMHA are now warranted.

1. Introduction

Immune-mediated hemolytic anemia (IMHA) is the most common immune-mediated hematologic disease in dogs (Swann and Skelly, 2013). Central to the pathogenesis of this disorder is the production of anti-erythrocyteautoantibodies. Antibody binding to erythrocytes in the bloodstream can lead to intravascular hemolysis, a process mediated by complement fixation. The complement system consists of a series of enzymatic plasma proteins, which are activated following complement protein interaction with antibody-coated cells. The end result of complement activation is the formation of the membrane attack complex (MAC), also known as C5B-9. The MAC complex binds to cell membranes near the site of antibody deposition and perforates the cell membrane, resulting in cell lysis.

People with paroxysmal nocturnal hemoglobinuria (PNH), a disease process that shares similarities with canine IMHA, suffer from bouts of intravascular hemolysis due to uncontrolled activation of the complement system (Brodsky, 2014). Recently, treatment of PNH has been revolutionized by the introduction of pharmaceutical complement inhibitors (Hillmen et al., 2007). Although the underlying pathobiology of PNH and IMHA is distinct, these diseases share the common final pathway of complement-mediated hemolysis. This similarity is the

Abbreviations: AUC, area under the curve; AUC_{last}, area under the plasma concentration-time curve from the time of dosing to the last measurable (positive) concentration; $AUC\text{-}INF_{obs}$, area under the plasma concentration-time curve from the time of dosing extrapolated to infinity; C1-INH, complement C1-esterase inhibitor; C_{max}, maximum observed plasma concentration; CL_{obs}, total body clearance; IMHA, immune-mediated hemolytic anemia; MAC, membrane attack complex; NOAEL, no adverse effect level; PK, pharmacokinetic; PNH, paroxysmal nocturnal hemoglobinuria; rC1-INH, recombinant C1-esterase inhibitor; T_{last}, time of last measurable (positive) concentration; T_{max}, time of maximum observed concentration; $T_{½}$, terminal half-life; VD_{obs}, volume of distribution based on the terminal phase

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premise for the present investigation, since it is hypothesized that complement inhibition could effectively treat canine IMHA.

C1-esterase inhibitor (C1-INH) is the primary *in vivo* inhibitor of complement activation in people (Schoenfeld et al., 2016). An *in vitro* screen of commercially available complement inhibitors suggested that a recombinant preparation of C1-INH is a very effective inhibitor of canine complement mediated hemolysis (Hernandez et al., 2018). C1-INH is commercially available in various preparations, including Berinert and Haegarda (CSL Behring LLC, Kankakee, IL), CINRYZE (ViroPharma Biologics, Inc., Lexington, MA), and Ruconest (Salix Pharmaceuticals, Inc., Raleigh, NC). The majority of these products (*e.g.* Berinert, Haegarda, and CINRYZE) are derived from human plasma and labelled for treatment and prophylaxis of hereditary angioedema in people. In contrast, Ruconest is a recombinant C1-INH (rC1-INH) product purified from the milk of transgenic rabbits and is indicated for the treatment of hereditary angioedema attacks in people. Previous studies evaluating the safety of this rC1-INH product have identified a no adverse effect level (NOAEL) of 625 IU/kg in dogs and rats (FDA filing memorandum STN: BL125495). Biological efficacy in dogs in the treatment of pathologies associated with excessive complement activation, has been documented at a dose of 500 IU (approximately 15–25 IU/kg) (Guerrero et al., 1993; Salvatierra et al., 1997). The potential utility of C1-INH in canine IMHA is further supported by evidence that C1-INH is active in both the fluid and solid phase (*i.e.* when the C1 is in its free soluble form or already bound to the cell membranes) meaning that the drug should also work during the clinical phase of the disease (Gigli et al., 1968).

In order to progress towards a clinical trial of C1-INH in dogs with IMHA, it should be confirmed that previously established safe doses of C1-INH in dogs achieve a plasma concentration capable of inhibiting canine complement activation. The immunogenicity of C1-INH is also a potential concern for clinical use, since C1-INH is a glycosylated peptide with the potential to elicit immune cell activation (Baker et al., 2010; Ratanji et al., 2014). This study aimed to fill these knowledge gaps by characterizing the pharmacokinetics of a single 500 IU IV dose of rC1-INH and to determine whether rC1-INH induces anti-drug antibody formation in healthy dogs.

2. Materials and methods

2.1. Drug

Single-dose vials of recombinant C1-INH (Ruconest, Pharming Group N.V., Leiden, Netherlands), each containing 2100 IU were purchased from a commercial wholesale company (ASD Healthcare, Frisco, TX). These vials were reconstituted per the manufacturer's instructions by adding 14 mL sterile water for injection to each vial to obtain a solution of 150 IU/mL.

2.2. Animals

Eight healthy institution-owned intact male Beagle dogs weighing between 6.5–10.6 kg were used for this study. All dogs were deemed healthy based on physical examination and through analysis of complete blood counts and serum chemistry panels. These dogs were cared for by licensed staff veterinarians and technicians according to institution animal care and use protocols, under local IACUC approval (Protocol 2016-0066). The rC1-INH preparation is available in single-dose vials, each containing 2100 IU, therefore each vial was sufficient to dose four 10 kg dogs with 50 IU/kg (IV). Thus, dogs were divided into two groups of four and each group was dosed on separate days. No adverse drug reactions were expected from administration of rC1-INH, however, all dogs were closely observed by veterinarians throughout the 24 h after drug administration. All dogs were permanently rehomed after use in this study.

2.3. Sample collection

On study days, central venous catheters were placed in external jugular veins to enable rC1-INH administration and to facilitate collection of multiple blood samples without repeated venipuncture. Dogs were sedated with intravenous butorphanol (0.2 mg/kg) and dexmedetomidine (2.5 μg/kg) (Zoetis Inc., Kalamazoo, MI, USA) to facilitate catheter placement. Triple lumen 7Fr × 20 cm wire-guided catheters (Arrow AK-15703 Multi-Lumen Central Venous Catheterization Kit, Teleflex, Morrisville, NC) were placed under aseptic conditions by experienced personnel using a modified Seldinger technique. Dogs received intramuscular atipamezole (0.17 mg/kg) (Zoetis Inc., Kalamazoo, MI, USA) after catheter placement, to reverse the dexmedetomidine effects, and were allowed to recover fully before rC1-INH administration. All blood samples were collected using a three-syringe technique into vacuum tubes (Vacutainer, BD and Co, Franklin Lakes, NJ, USA) containing either 3.2% sodium citrate (for rC1-INH concentration measurement), K_2-EDTA (for complete blood counts) or no-additive (for antibody testing).

Since the half-life of rC1-INH is ~20 min and 2.5 h, in rats and people respectively (EMA/CHMP/450053/2010, FDA filing memorandum STN: BL125495), the sampling protocol employed assumed the half-life in dogs would fall somewhere between these two values. Baseline (time 0) samples were collected immediately prior to drug administration. The rC1-INH was given intravenously over 5 min by slow push and blood samples were then collected at 10, 20, 40, 60, 80, 100, 120, 240, 360, 480, 600, 720, 960, and 1440 min after drug administration. An additional blood sample was collected from each dog 14 days after drug administration for detection of antibody formation. The 14-day time point was selected as an adequate time for a primary immune response to have occurred (Ademokun and Dunn-Walters, 2001).

2.4. Serum rC1-INH concentration measurement

Serum concentrations of rC1-INH were measured using a commercial functional ELISA (C1-INH ELISA, MicroVue, Quidel, San Diego, CA, USA). In this assay, both biotinylated and active C1s (the target of C1-INH) react with the standards and samples, forming covalent complexes. These rC1-INH-C1s-biotin complexes react with an avidin-coated plate. Following appropriate wash steps, the complexes are detected with a conjugated anti-C1-INH antibody such that color development is proportional to the amount of rC1-INH in the sample. A standard curve using 5 dilutions of rC1-INH and logistic regression was used to convert the absorbance values into IU/mL of rC1-INH.

2.5. Hematology and coagulation analyses

Complete blood counts were performed on EDTA anticoagulated samples using a benchtop analyzer (Advia 2120, Siemens, Washington, D.C., USA). Plasma D-dimer concentrations were measured using a quantitative, turbidimetric immunoassay, with a human D-dimer calibration standard and controls (HemosIL D-dimer and D-dimer Calibrator, Instrumentation Laboratory, Lexington, MA, USA).

2.6. Anti rC1-INH antibody responses

Western blot analysis, using aliquots of the drug (peptidic in nature) as the target antigen was used to detect anti-drug antibodies in the serum of dogs administered rC1-INH. Serum samples for antibody testing were collected before, and 14 days after drug administration. A rabies vaccine (IMRAB 3, Merial Limited. Duluth, GA, USA) was used as an assay control target antigen for method validation purposes, since all the dogs were immunized against rabies using this vaccine. A no-primary antibody control was also included. A 1:500 dilution of rC1-INH in Laemmli Buffer containing β-mercaptoethanol (Bio-Rad

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