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Regular Article

Clinical Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of the Novel Factor Xa Inhibitor DY-807f in Healthy Volunteers

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ABSTRACT

Introduction: Since Vitamin K antagonists are associated with various limitations such as narrow therapeutic window, slow onset and offset of effect, and numerous interactions with food and drugs, new oral anticoagulants targeted to inhibit thrombin or factor Xa (FXa) have been developed. DY-807f is a highly selective, reversible and orally bioavailable FXa inhibitor.
Objectives: This article describes a first-in-human study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of single ascending oral doses of the novel direct FXa inhibitor DY-807f in healthy males.
Methods: a placebo-controlled, single-blinded, randomized, single ascending dose study with 84 subjects (10, 30, 60, 120, 240, and 360 mg). Effects of food and formulation (tablet vs solution) on bioavailability of 60 mg were also assessed as a crossover design.
Results: DY-807f doses were safe and well-tolerated with no dose-dependent increase in adverse events up to 360 mg. Pharmacokinetics profiles were consistent across doses with rapid absorption, biphasic elimination, and terminal elimination half-life of 10.5 to 12.4 hours. Coagulation parameters (Activated Partial Thromboplastin Time (aPTT) and Prothrombin Time (PT)) were linearly correlated with plasma DY-807 (free base of DY-807f) concentrations (correlation coefficient: 0.786 for aPTT, 0.945 for PT).
Conclusions: Single doses of DY-807f are safe and well-tolerated up to 360 mg with predictable pharmacokinetic and pharmacodynamic profiles.

Introduction

Vitamin K Antagonists (VKAs) such as warfarin are currently the most widely used oral anticoagulants for the prevention and treatment of venous thromboembolism and prevention of embolism in patients with atrial fibrillation [1]. However, management of VKAs is complicated due to their narrow therapeutic index, slow onset and offset of effect, and numerous interactions with food and drugs. Therefore, frequent monitoring and dose adjustment are essential to manage patients on VKA therapy and these limitations contribute to underuse of VKAs in patients requiring long-term anticoagulation [2,3]. The need for parenteral administration of heparin and its derivatives is an additional considerable obstacle to the long-term use of these agents. As outlined above, there is a need for new oral anticoagulants with sufficiently predictable pharmacokinetics, pharmacodynamics and no need for regular monitoring or dosage adjustment for long-team clinical use.

To address the unmet medical needs, oral thrombin inhibitors and oral factor Xa (FXa) inhibitors have recently been developed such as dabigatran [4], rivaroxaban [5], edoxaban [6] and apixaban [7]. Factor Xa is an integral component of the coagulation cascade and is generated via both the intrinsic and extrinsic pathways. It is also the rate-limiting step for the propagation of thrombin generation. Thus, direct inhibition of FXa with small, specific molecules is becoming an increasingly attractive antithrombotic strategy [8].

DY-807f is a novel small molecule that specifically and reversibly inhibits FXa and can be orally administered. Preclinical studies have demonstrated excellent oral availability and potential as an antithrombotic agent in animals (in-house data). This study was a first-in-human study conducted to evaluate clinical safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of ascending single oral dose of DY-807f in healthy subjects.

Materials and Methods

Study Design

This was a phase I, single-blinded, randomized, placebo-controlled, single ascending oral dose study to assess safety, tolerability,

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Pharmacokinetics (PK) and Pharmacodynamics (PD) of the novel FXa inhibitor DY-807f conducted in healthy male volunteers. The study consists of two protocols, the first protocol was low dose study with 36 subjects and consecutively the second protocol was high dose study with 48 subjects. Subjects in both the low- and high-dose studies were administered DY-807f in ascending dose order. There was at least 7 days between dose escalation which proceeded after review of safety, PK and PD data of each preceding dose. All subjects returned to the clinical research unit 5 to 7 days after receiving their final dose for clinical safety assessments (post-study). In addition, two sub-studies in the 60 mg single-administration dose groups were conducted as crossover designs to assess the effect of food and formulation on PK and PD in the low- and high-dose study, respectively.

Low-Dose Study

Subjects in the low-dose study received 10 mg, 30 mg, or 60 mg DY-807f or placebo in tablet form while fasted (n = 12 in each group). In the groups receiving 10 mg through 60 mg, ten subjects received drug and two subjects received matching placebo tablets.

Subjects in the 60 mg group participated in the food effect study and were randomized to receive DY-807f or placebo with and without food and then crossed over to receive the alternate treatment after 6 days or later. The high-fat meal was prepared in accordance with the Food and Drug Administration Guidance for industry [9].

High-Dose Study

The high-dose study was initiated after analysis of the low-dose study. Subjects in the high-dose study received 60 mg, 120 mg, 240 mg, or 360 mg DY-807f or placebo in tablet form while fasted (n = 12 in each group). In the group of 60 mg, 10 subjects received the drug and 2 subjects received matching placebo. In the groups that received 120 to 360 mg DY-807f, the ratio of drug to placebo was 3:1 (n = 12 in each group).

Subjects in the 60 mg group participated in the sub-study to assess the bioavailability of 60 mg DY-807f in solution or tablet form. Subjects were randomized to solution or tablet (DY-807f n = 10, placebo n = 2) and crossed over to receive the alternate formulation after 6 days or later. The placebo solution included denatonium benzoate (Bitrex) to mask the taste profile of DY-807f in solution.

These studies were approved by the Institutional Review Board of Covance Clinical Research Unit, Ltd. and carried out in accordance with the Declaration of Helsinki. All subjects gave written informed consent to participate in the study.

Study Population

Healthy Caucasian males between the ages of 18 and 55 years with a body mass index (BMI) between 19 and 29 kg/m^2 were eligible for enrollment.

Major exclusion criteria included drug or alcohol abuse; use of drugs known to alter drug metabolism within the preceding 30 days; any prescription medication in the preceding 14 days; donating blood/plasma within the past 3 months or more than 1500 mL blood/ plasma loss within the preceding 12 months; history of allergic reactions to drugs or anticoagulants or ongoing allergic disease (except for non-active seasonal allergies); genetic blood clotting factor deficiency; blood pressure more than 150/90 mm Hg or less than 100/50 mm Hg and pulse rate more than 90 beats per minute or less than 40 beats per minute, respectively; dental extraction or surgery in the preceding 4 weeks; history of frequent epistaxis; peptic ulcer or gastro-esophageal reflux within the past 2 years; and other general clinically significant disorders or illnesses which could confound the interpretation of the safety and tolerability of DY-807f in this study.

For the duration of the study the consumption of caffeine, grapefruit juice and alcohol were forbidden and subjects were limited to 5 or fewer cigarettes per day, given the known effects of these agents upon coagulation [1]. Standard meals were provided for all subjects while resident in the research unit.

Drug

DY-807f tablets (10 mg and 60 mg), matching placebo tablets, and DY-807f powder for preparation of oral solution were manufactured by Daiichi Sankyo Co, Ltd. (Tokyo, Japan) in accordance with Good Manufacturing Practice and repackaged and labeled by Clinical Trial Services, Craigavon, Northern Ireland.

PK

Blood samples (1 x5 mL) were collected from forearm vein(s) into lithium heparin tubes (Becton Dickinson UK Ltd., Oxford, UK) at serial time points from 0 to 48 hours after DY-807f administration and centrifuged at 1500 g for 10 minutes at 4 °C within 30 minutes. Plasma was separated into two polypropylene tubes and stored in the dark at −20 °C within 1 hour of collection and samples were transferred on dry ice.

Urine was collected from 0 to 48 hours after DY-807f administration into pre-weighed polyethylene containers and stored at 2-8 °C until weighed. After weighing, two 4 mL samples were aliquoted into polypropylene containers and stored at −20 °C in the dark within 1 h of collection. Two additional 4 mL samples were diluted 100-fold with control human urine, transferred into polypropylene containers and stored in the dark at −20 °C. A nominal value for specific gravity of 1.018 was used to calculate urine volume. All urine samples were transferred on dry ice.

Blood samples (1 x 5 mL) for plasma protein binding were collected from forearm vein(s) into lithium heparin tubes at 3 and 24 hours after administration of DY-807f 120 mg. Within 1 hour of collection, blood samples were centrifuged at 1500 g for 10 minutes at 4 °C. Plasma was separated into two polypropylene tubes and stored in the dark at −20 °C within 1 hour of collection and samples were transferred on dry ice. Protein binding was determined by ultracentrifugation (45 000 rpm [approximately 200 000 g] for 20 hours at 40 °C) of plasma samples to separate the supernatant. The concentration of DY-807 (the free base of DY-807f) was measured in the resulting supernatant. The difference between the plasma (total DY-807) and supernatant (unbound DY-807) concentrations provided the estimates of protein binding.

DY-807 in plasma and urine was quantified by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) by Quotient Bioresearch Ltd, Fordham, UK. DY-807 and the stable-labeled internal standard (IS) (DY-807-d6,) were extracted from 0.2 mL of human plasma or urine using 3 mL of methyltertiarybutyl ether. The extract was evaporated and reconstituted with 0.2 mL of 0.1% formic acid /acetonitrile (3:1, v/v). Five microliter (μL) for plasma and 2 μL for urine were injected onto a Atlantis dC18 column (2.1 mm i.d. x 20 mm, Waters, Hertfordshire, UK), which was connected to an API 3000 mass spectrometer (Applied Biosystems-MDS/Sciex, Warrington, UK) equipped turbo-ionspray with positive ion mode. DY-807 and IS analytes were eluted using a mobile phase composed of 0.1% formic acid /acetonitrile (3:1, v/v). Flow rate was 0.5 mL/min (2:1 split into the MS). The multiple-reaction-monitoring transitions monitored applied were 548.5 → 376.0 and 554.5 → 382.4, for DY-807 and IS, respectively. The lower limit of DY-807 detection in plasma and urine was 1 and 20 ng/mL, respectively.

Noncompartmental PK analysis for plasma and urine DY-807 concentrations was performed using WinNonlin Enterprise Version 4.0.1. Statistical analyses of these parameters included analysis of variance (ANOVA) and nonparametric methods. $AUC_{0-\tau}$ was calculated by the trapezoidal method to the last time point. $AUC_{0-\infty}$ was estimated by addition of extrapolation using the last plasma concentration/λ added to $AUC_{0-\tau}$. The apparent terminal elimination half-life ($t_{1/2}$) was