Thrombosis Research 127 (2011) 292–298

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Safety Assessment and Pharmacodynamics of a Novel Ultra Low Molecular Weight Heparin (RO-14) in Healthy Volunteers – A First-Time-In-Human Single Ascending Dose Study☆

Salvador Rico [a,b,*], Rosa Maria Antonijoan [a,b], Ignasi Gich [a,b], Montserrat Borrell [c], Jordi Fontcuberta [c], Mayte Monreal [d], Javier Martinez-Gonzalez [d], Manel J. Barbanoj [a,b,1]

[a] Centre d'Investigació de Medicaments, Institute of Biomedical Research (IIB Sant Pau), Barcelona, Spain
[b] Department of Pharmacology and Therapeutics, Autonomous University of Barcelona, Spain
[c] Hemostasis and Thrombosis Unit, Department of Hematology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
[d] Medical Department, Laboratorios Farmacéuticos Rovi, S.A., Madrid, Spain

ARTICLE INFO

Article history:
Received 10 August 2010
Received in revised form 14 December 2010
Accepted 15 December 2010
Available online 22 January 2011

Keywords:
RO-14
ultra low molecular weight heparin
pharmacodynamics
safety
anti-FXa
anticoagulants

ABSTRACT

Introduction: RO-14 is a novel ultra low molecular heparin. The purpose of this study was to evaluate the safety and pharmacodynamic profile of RO-14 in healthy males.
Materials and methods: We conducted a two-stage, single-center, open-label, randomized study. Two cohorts of 6 volunteers were randomly assigned to 12 single, ascending subcutaneous doses (1750-19950 IU of anti-FXa activity) in an alternating crossover fashion. Safety was assessed by spontaneous/elicited adverse events, medical examination and laboratory tests. Anti-FXa activity and anti-FIIa activity were assessed throughout the 24 hours after dosing. Dose proportionality and linearity of the anti-FXa activity were evaluated.
Results: All doses were well tolerated and there were no bleeding events. At the lowest dose, anti-FXa activity A_{max} was 0.16 (±0.02) IU/mL and $AUC_{0\text{-}24}$ was 1.11 (±0.24) IU*h/mL, At the highest dose anti-FXa activity A_{max} was 1.67 (±0.15) IU/mL; $AUC_{0\text{-}24}$ was 21.48 (±4.46) IU*h/mL and t½ was 8.05 h. Mean T_{max} (all doses) was 2.86 (±0.39) h. RO-14 showed proportional and linear pharmacodynamics [normalized A_{max} among doses ($p = 0.594$) and normalized $AUC_{0\text{-}24}$ ($p = 0.092$), correlations between A_{max}-dose ($R^2 = 0.89$, $p < 0.001$) and $AUC_{0\text{-}24}$-dose ($R^2 = 0.86$, $p < 0.001$)]. Anti-FIIa activity was below the detection limit (0.1 IU/ml) at all dose levels. No clinically significant changes were observed in the platelet count, APTT, PT, TT, fibrinogen and antithrombin.
Conclusions: In this phase I study, RO-14 exhibited a good safety profile, anti-FXa activity for either prophylaxis or treatment of venous thromboembolism, linear pharmacodynamics, a longer elimination half-life than currently marketed low molecular weight heparin and no anti-FIIa activity.

Introduction

Venous thromboembolism (VTE), encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE), is one of the major common endpoints in human disease and a frequent complication among hospital inpatients [1]. Since their development, 2 decades ago, low molecular weight heparins (LMWH) have addressed some of the disadvantages shown by unfractioned heparin (UFH). LMWHs produce a more predictable anticoagulant response than UFH, reflecting their better bioavailability, longer half-life, and dose-independent clearance [2]. These characteristics have eliminated the need for monitoring in most cases and reduced the risk of bleeding [2,3]. Due to their advantages, LMWHs have largely replaced UFH for many indications[3] and are nowadays standard care for the prevention and treatment of VTE [4,5].

Several LMWHs have been developed (e.g enoxaparin, dalteparin, tinzaparin, bemiparin, etc.). All have slightly different properties and

Abbreviations: APTT, Activated partial thromboplastin time; AE, Adverse event; AEMPS, Agencia Espanola de Medicamentos y Productos Sanitarios; AT, Antithrombin; $AUC_{0\text{-}24}$, Area under the anti-FXa activity-time curve in the 24 hours after drug administration; $AUC_{0\text{-}\infty}$, Area under the anti-FXa-time curve extrapolated to infinity; Cl/F, Clearance; DVT, Deep vein thrombosis; ECG, Electrocardiogram; t ½, Elimination half-life; FOBT, Fecal occult blood test; FTIH, First-Time-In-Human; GMP, Good manufacturing practice; A_{max}, Maximum anti-FXa activity; MRSD, Maximum recommended starting dose; NOAEL, No Observable Adverse Effect Level; PT, Prothrombin time; PE, Pulmonary embolism; T_{max}, Time to reach maximum anti-FXa activity; TT, Thrombin time; VTE, Venous thromboembolism; Vd/F, Volume of distribution; ULMWH, Ultra Low Molecular Weight Heparin; UFH, Unfractioned heparin.

☆ The study was presented in part at the 22nd Congress of the International Society on Thrombosis and Haemostasis, Boston, USA, 15-16 July, 2009

* Corresponding author. Centre d'Investigació de Medicaments, IIB-Sant Pau, Hospital de la Santa Creu i Sant Pau, Sant Antoni Maria Claret 167, Barcelona 08025, Spain. Tel.: +34 93 553 7199; fax: +34 93 553 7864.
E-mail addresses: sricoa@hotmail.com, sricoa@santpau.cat (S. Rico).

[1] In memoriam – Manel J. Barbanoj passed away in the company of his family and friends on December 12th, 2010. We have lost a great mentor, an outstanding researcher and a wonderful friend. May he rest in peace.

0049-3848/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.thromres.2010.12.009

licenses for different risk situations [6]. Each LMWH is a unique chemical entity and the results of clinical trials or pharmacodynamic studies cannot be extrapolated from one product to another [7–9].

Despite the recent development of new options in antithrombotic therapy (e.g. orally administered direct factor Xa inhibitors and direct thrombin inhibitors), there is still room for improvement [5]. One of the lines of research that has received attention is the development of LMWHs with an optimized profile. RO-14 is a new ultra low molecular weight heparin (ULMWH) currently under clinical development. The main theoretical advantage is conferred by the lower mean molecular weight, by the enhanced anti-FXa activity that has been suggested to be involved with an improved anti-thrombotic efficacy [10], the potential longer elimination half-life and by the larger anti-FXa:FIIa activity ratio. In regards to this last supposed advantage, it is noteworthy that there seems to exist a larger safety margin with anti-FXa inhibition with respect to bleeding in comparison to inhibition of thrombin [11–13].

As part of the drug development program, the aim of this first-time-in-humans (FTIH) study was to provide essential information about the safety, tolerability and preliminary pharmacodynamics of RO-14.

Materials and methods

Study design

This was a two-stage, two cohort, alternating crossover, single-center, open-label, controlled, randomized, single ascending dose study to evaluate the safety, tolerability and preliminary pharmacodynamics of RO-14 in healthy male volunteers. The protocol was approved by the Hospital de la Santa Creu i Sant Pau - Clinical Research Ethics Committee and the Spanish Drug Agency (AEMPS). The study was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice guidelines. All subjects gave written informed consent to participate in the study and were paid for their collaboration. The study is registered at clinicaltrials.gov (ref. #NCT00629733).

Study drug

The study drug is the sodium salt of a novel ULMWH denominated RO-14 (Laboratorios Farmaceúticos Rovi, S.A., Spain). RO-14 is obtained by selective chemical despolyimerization of UFH in a non-aqueous medium, following a beta-elimination method [14].

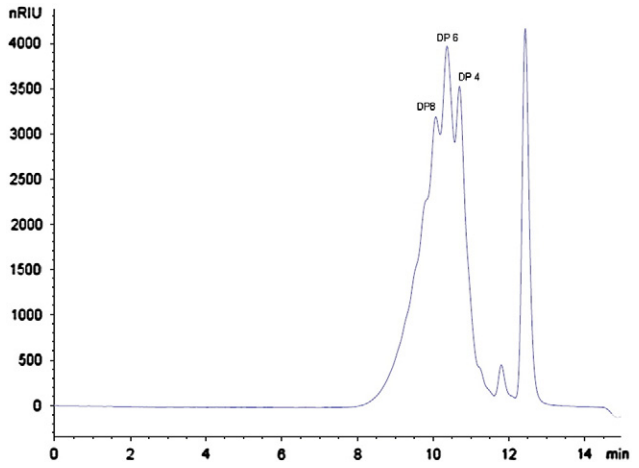

Fig. 1. GPC chromatogram of RO-14: RO-14 has a mean molecular weight of 2,200 Da is composed of 25-40% oligosaccharides with a mean MW lower than 2000 Da, 40-75% oligosaccharides with MW between 2000 and 6000 Da and less than 15% oligosaccharides with a MW higher than 6000 Da (DP: Degree of Polymerization).

RO-14 has an anti-factor Xa in vitro activity between 80 and 140 IU/mg and an anti-factor IIa in vitro activity lower than or equal to 7 IU/mg. RO-14 has an anti-factor Xa/anti-factor IIa ratio higher than 20.

RO-14 is composed of mixtures of fragments of heparin or oligosaccharides (Fig. 1). Its mean molecular mass by weight is between 1,800 and 3,000 Da. Its weight-average molecular weight ranges with a mean value of 2,200 Da. Its composition is the following:

- from 25 to 40% of oligosaccharides of molecular mass under 2,000 Da;
- from 40 to 75% of oligosaccharides of molecular mass between 2,000 and 6,000 Da; and
- less than 15% of oligosaccharides of molecular mass over 6,000 Da.

The investigational product was manufactured according to current good manufacturing practice (GMP) procedures and dispensed in individually-sealed boxes, labeled according to a randomization code. The randomization code was generated with SPSS Statistics 17.0 by a person not involved in the conduct of the study.

RO-14 was supplied in 0.5 mL pre-filled syringes containing sterile and endotoxin-free aqueous solution for subcutaneous injection. The following RO-14 strengths were used: 1750 IU anti-FXa/0.2 mL, 2450 IU anti-FXa/0.2 mL, 3500 IU anti-FXa/0.2 mL, 4550 IU anti-FXa/0.2 mL, 5600 IU anti-FXa/0.3 mL, 6650 IU anti-FXa/0.3 mL, 7700 IU anti-FXa/0.4 mL, 10150 IU anti-FXa/0.8 mL, 12600 IU anti-FXa/0.8 mL, 15050 IU anti-FXa/0.8 mL, 17500 IU anti-FXa/0.8 mL and 19950 IU anti-FXa/0.8 mL. Doses of 10150 IU and above were divided evenly in 2 syringes containing 0.4 mL each. An International Unit of anti-FXa activity is defined according to the standards established by the 1st International Standard for Low Molecular Weight Heparins (National Institute for Biological Standards and Control, United Kingdom) [15].

Maximum recommended starting dose

The maximum recommended starting dose (MRSD) in this FTIH study was determined following the Food and Drug Administration (FDA) guideline entitled "Estimating the Maximum Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers" [16].

Given that the No Observable Adverse Effect Level (NOAEL) in the most sensitive species (i.e. Beagle dog) derived from toxicology studies was 500 IU anti-FXa/kg SC [17], the MRSD was calculated to be 27 IU anti-FXa/kg. This was further rounded to 25 IU anti-FXa/kg for practical purposes.

Subjects and study conduct

Healthy male subjects, aged between 18 and 45 years of age and weighing 65-75 kg, were screened for inclusion in the study. Subjects had to be healthy as judged by medical history, physical examination, vital signs, electrocardiogram (ECG) and clinical laboratory results. They were excluded if they were smokers of more than 10 cigarettes a day, and if they had known history of hypersensitivity to drugs, coagulation disorders (e.g. von Willebrand disease, hemophilias), conditions with an increased bleeding risk (eg. peptic ulcer, hemorrhoids, acute gastroenteritis, or sensitivity to nasal bleeding), abnormal coagulation tests, urinalysis positive for hematuria, positive fecal occult blood test (FOBT), positive serology for hepatitis B, C, or HIV virus, trauma or surgery in the 6 months prior to the study, any medication 15 days prior to the trial, or history of drug abuse or chronic disease. Importantly, subjects were known not to have taken aspirin or aspirin-containing medications in the 10 days before the study.

The study was divided into two stages, A and B. Stage A assessed 6 ascending dose levels of RO-14 (1750, 2450, 3500, 4550, 5600, 6650 IU anti-FXa), each administered as a single subcutaneous injection in the abdominal region. After these 6 doses, it was deemed