Thrombosis Research 131 (2013) 425–435

Contents lists available at SciVerse ScienceDirect

Thrombosis Research

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

Regular Article

Antithrombotic activity of HY023016, a novel Dabigatran prodrug evaluated in animal thrombosis models

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ARTICLE INFO

Article history:
Received 1 December 2012
Received in revised form 15 February 2013
Accepted 1 March 2013
Available online 25 March 2013

Keyword:
HY023016
Novel Dabigatran prodrug
Anti-thrombotic activity
Tissue factor expression
Bleeding time

ABSTRACT

Introduction: Thrombin is a multifunctional trypsin-like serine protease that plays key roles in coagulation and thrombogenesis. HY023016, a novel Dabigatran prodrug, is an oral direct thrombin inhibitor. The purpose of this study was to compare the anti-thrombotic activities and haemorrhagic effects of HY023016 with Dabigatran etexilate and tetramethylpyrazine in several animal thrombosis models.

Methods: To investigate drug exposure, liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used to determine the pharmacokinetic profile of HY023016. After single intragastric administrations of HY023016, Dabigatran etexilate or tetramethylpyrazine, the anti-thrombotic activities were evaluated through rabbit jugular vein thrombosis model, rat inferior vena cava thrombosis model, *ex vivo* rabbit platelet aggregation assay, *in vivo* rabbit coagulation assay, and direct thrombin binding assay. Meanwhile, we evaluated the effect of HY023016 on expression of tissue factor (TF) by RT-PCR. Rabbit cuticle bleeding assay and mouse tail bleeding assay were applied to evaluate the effects of HY023016 on haemorrhage.

Results: Pharmacokinetic parameters indicated that HY023016 can convert to Dabigatran and tetramethylpyrazine. Our studies showed that HY023016 was able to significantly inhibit thrombus formation in a dose-dependent manner in rabbit and rat models ($P < 0.05$). Similarly, it was able to dose-dependently inhibit thrombin- or ADP-induced platelet aggregation, prolonging the activated partial thromboplastin time (APTT) and prothrombin time (PT), inhibiting the activity of thrombin and inhibiting thrombin- or ADP-induced expression of TF ($P < 0.05$ or 0.01). Dabigatran etexilate was also able to dose-dependently and significantly inhibit thrombus formation ($P < 0.01$) but was unable to affect ADP-induced platelet aggregation and expression of TF. In contrast, tetramethylpyrazine could only exhibit mild antithrombotic activity compared with HY023016 and Dabigatran etexilate ($P < 0.05$). HY023016 could prolong bleeding time ($P < 0.001$), but the prolongations were significantly less than Dabigatran etexilate ($P < 0.05$).

Conclusion: HY023016 showed thrombosis-inhibition activities comparable to those of Dabigatran etexilate, but better than those of tetramethylpyrazine. The attendant bleeding risk of HY023016 was lower than Dabigatran etexilate in rabbits and mice.

Introduction

There is no doubt that thrombosis is the most common cause of death in the modern world, and whether through venous thromboembolism, myocardial infarction or stroke, ultimately involves the generation of thrombus [1]. An ideal antithrombotic drug would possess the following characteristics: oral administration, no requirements for routine coagulation monitoring and dosage adjustments, wide therapeutic window, rapid onset of action, predictable pharmacokinetics and pharmacodynamics, minimal interactions with foods and other drugs, ability to inhibit free and clot-bound coagulation factors, low non-specific binding, availability of an antidote, no unexpected toxicities, and acceptable costs [2–4]. Because thrombin plays a central role in thrombotic diseases [1], several oral direct thrombin inhibitors

Abbreviations: LC-MS/MS, liquid chromatography-tandem mass spectrometry; APTT, activated partial thromboplastin time; PT, prothrombin time; TF, Tissue factor; CMC-Na, Sodium carboxymethylcellulose; DMSO, dimethyl-sulfoxide; ADP, adenosine diphosphate glucose pyrophospheralase; RT-PCR, reverse transcription-polymerase chain reaction; PRP, platelet-rich plasma; PPP, platelet-poor plasma; OD, optical density; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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http://dx.doi.org/10.1016/j.thromres.2013.03.004

have been developed, such as Dabigatran etexilate, Ximelagatran and so on [5,6]. After Ximelagatran was withdrawn from the market because of its hepatotoxicity, Dabigatran etexilate became the only oral direct thrombin inhibitor approved for sale on the market.

As a potential antithrombotic drug, Dabigatran etexilate has been used for prevention of venous thromboembolism in major orthopedic surgeries, treatment of venous thromboembolism, and prevention of stroke in atrial fibrillation [7–9]. Through further clinical research, several problems with the use of Dabigatran etexilate in antithrombotic therapy have emerged. Without any complementary antiplatelet therapies, Dabigatran etexilate could increase the risk of myocardial infarction and the levels of platelet activities [10]. Dabigatran etexilate also cannot down-regulate the level of platelet-monocyte aggregates, platelet-granulocyte aggregates and TF mRNA, which is induced by ADP [11]. Based on the reasons described above, a series of novel Dabigatran prodrugs have been synthesized. After pharmacodynamic screening *in vitro*, we found that HY023016 has excellent anti-thrombotic activity [12]. HY023016 has two nucleuses - Dabigatran and tetramethylpyrazine (Fig. 1). Tetramethylpyrazine, as a major active component of the herbal medicine Chuanxiong (*Ligusticum wallichii Franchat*), displays mild antiplatelet activity. HY023016 is expected to be able to overcome the defects of Dabigatran etexilate and be able to exhibit the equivalent anti-thrombotic activity.

The purpose of the present study was to compare the antithrombotic activity of HY023016 with Dabigatran and tetramethylpyrazine in several animal venous thrombosis models. We simultaneously measured the effects of HY023016, Dabigatran etexilate and tetramethylpyrazine on the expression of tissue factor. We also determined the effects of HY023016, Dabigatran etexilate and tetramethylpyrazine on bleeding time in the mouse tail bleeding model and investigated its potential safety.

Materials and Methods

Materials

HY023016, Dabigatran etexilate and tetramethylpyrazine were obtained from the Department of Medicinal Chemistry, China Pharmaceutical University. Sodium carboxymethylcellulose (CMC-Na), dimethyl-sulfoxide (DMSO) and $FeCl_3$ were purchased from Shanghai Reagent Station (Shanghai, China). Ethyl p-aminobenzoate and citrate tribasic dihydrate were purchased from Shanghai Qingxi Chemical Technology Co., Ltd. (Shanghai, China). Sodium pentobarbital was purchased from China National Pharmaceutical Group Corporation (Shanghai, China). Polidocanol was purchased from Shaanxi Tianyu Pharmaceutical Co., Ltd. (Xian, China). Activated partial thromboplastin time (APTT) assay kit, prothrombin time (PT) assay kit and Adenosine Diphosphate Glucose Pyrophospheralase (ADP) were purchased from Nanjing Jiangcheng Bioengineering Institute (Nanjing, China). Thrombin was purchased from Hunan Yige Pharmaceutical Co., Ltd. (Xiangtan, China). D-Phe-Pro-Arg-pNA was purchased from Univ-Bio (Shanghai, China). One Step RNA PCR Kit was purchased from Takara BIO (Dalian, China). All other chemicals used in the experiments were analytical grade.

Animals

Sprague–Dawley rats (180–220 g), New Zealand White rabbits (2.0-2.5 kg) and ICR mice (18–22 g) were provided by the Laboratory Animal Center of China Pharmaceutical University. All animals were in a well-ventilated animal house kept at 25 °C ± 5 °C under 12 hours light and dark cycle. The animals were allowed to readjust to the new housing environment for 1 week before the experiment. All procedures were conducted in accordance with the Guidelines on the Care and Use of Laboratory Animals (Chinese Council on Animal Research and the Guidelines of Animal Care). The study was approved by the Ethical Committee of China Pharmaceutical University.

Preliminary Pharmacokinetic Study in Rats

HY023016 was dissolved in 0.5% CMC-Na with 0.4% DMSO. After fasting for 16 hours, HY023016 (20 mg/kg) was administered via oral gavage (8 rats per group). Blood samples were collected 0.5 h before drug administration and again at 0.167, 0.25, 0.5, 1, 1.5, 2, 4, 6 and 24 h post-administration. Venous blood samples (approximately 0.2 ml) were collected from orbital venous plexus and centrifuged immediately

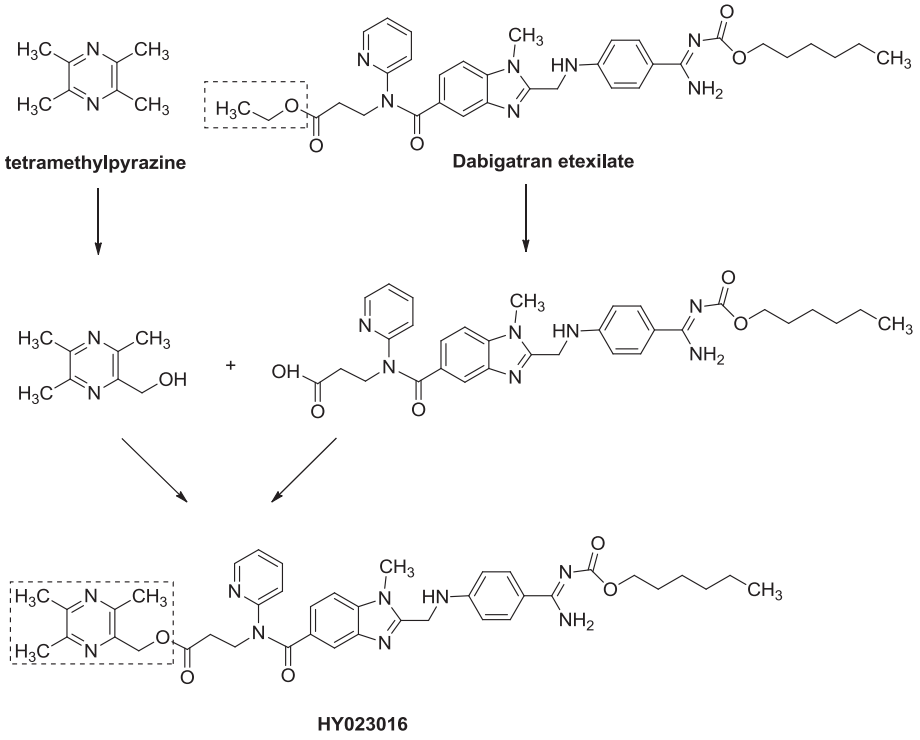

Fig. 1. Chemical structure of HY023016.