Thrombolysis with rt-PA under Rivaroxaban Anticoagulation in a Hypertensive Rat Model of Intraluminal Middle Cerebral Artery Occlusion

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Background: The aim of this study was to assess the risk and the threshold of hemorrhagic transformation (HT) after treatment with recombinant tissue plasminogen activator (rtPA) under the novel oral anticoagulant, rivaroxaban. *Methods:* Fifty-three spontaneous hypertensive rats were used in this study. We performed transient middle cerebral artery occlusion for 270 minutes. Placebo, 10 mg/kg or 20 mg/kg rivaroxaban were administered via a stomach tube 180 minutes after induction of ischemia, and rtPA (10 mg/kg) was administered just before reperfusion. Ninety minutes after rivaroxaban administration we measured the rivaroxaban plasma concentration and prothrombin time (PT). HT volume was assessed by hemoglobin spectrophotometry. Additionally, infarct volume, IgG leakage volume, and neurological outcome were assessed. *Results:* Rivaroxaban plasma concentration and PT increased in a dose dependent manner but were lower than human peak levels after a once-daily dose of 20 mg rivaroxaban. HT volume increased after treatment with 20 mg/kg rivaroxaban compared with placebo treated controls or those treated with 10 mg/kg rivaroxaban (26.5 ± 5.4, 26.8 ± 8.7, and 41.4 ± 12.6 μL in placebo, 10 mg/kg, and 20 mg/kg treated groups, respectively; $P < .05$). *Conclusions:* Our results suggest that even at therapeutic plasma concentrations, rivaroxaban may increase the risk of HT after thrombolysis in some conditions, such as hypertension and/or a prolonged ischemic period.

Key Words: Cerebral infarction—Fibrinolysis—Oral anticoagulants—Rat

Introduction

Rivaroxaban is a direct factor Xa inhibitor and is one of the novel oral anticoagulants for preventing stroke in patients with atrial fibrillation. The ROCKET AF study revealed that once-daily fixed-dose rivaroxaban was noninferior to warfarin, even on an intention-to-treat analysis, for the prevention of stroke and systemic embolism in patients with atrial fibrillation.[1] Additionally anticoagulation with rivaroxaban was associated with significantly less primary intracerebral hemorrhagic compared with warfarin.[1] In the study, although it was less common, a certain number of patients developed embolic stroke in both the rivaroxaban and warfarin treated groups.[1] To date, the optimal management of patients with ischemic stroke in patients under anticoagulants at the time of onset has not been established. Because of concerns of increasing the risk of hemorrhagic transformation (HT), the current guidelines recommend the exclusion of patients currently undergoing treatment with direct factor Xa inhibitors with elevated sensitive laboratory tests, from intravenous thrombolysis with recombinant tissue-plasminogen activator (rtPA).[2] On the other hand, several preclinical studies using cerebral ischemic models have

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reported that rivaroxaban does not increase hemorrhage after thrombolysis with rtPA[3,4] and several case reports have reported thrombolysis in patients undergoing rivaroxaban treatment without complications.[5-9] However, the risk of HT after thrombolysis during treatment with rivaroxaban is not fully understood and useful laboratory tests to determine the safety of thrombolysis with rtPA remains to be established in patients with undergoing rivaroxaban treatment.

The aim of this study was to evaluate the risk of rtPA-associated HT in a rat model of focal cerebral ischemia undergoing treatment with different dosages of rivaroxaban compared with control rat.

Materials and Methods

All experimental protocols were approved by the Institutional Committee on Animal Research, and were conducted in accordance with the National Institutes of Health guidelines for animal use, and the care and use of laboratory animals.

General Preparation and Induction of Cerebral Ischemia (Fig 1)

Adult male, spontaneous hypertensive rats (SHR/Hoshino, SLC, Japan, 12 weeks-13 weeks of age, body weight 250 grams-300 grams, total n = 53) were used, because more obvious HT was observed in these rats compared with Sprague Dawley rats in our preliminary investigations. After induction of anesthesia with 3% halothane in a gas mixture of 70% nitrous oxide and 30% oxygen, anesthesia was maintained with 1% halothane via a facemask. The femoral artery was cannulated for continuous monitoring of arterial blood pressure and to obtain blood O_2 and CO_2 partial pressure, pH, prothrombin time (PT), and rivaroxaban plasma concentration. The other femoral vein was cannulated for rtPA administration. Rectal temperatures were monitored and maintained at 37 ± .5°C using a feedback-controlled heating system.

To mimic the most severe clinical situation, we applied 270 minutes of middle cerebral artery occlusion (MCAO) using an intraluminal suture, as described previously.[10]

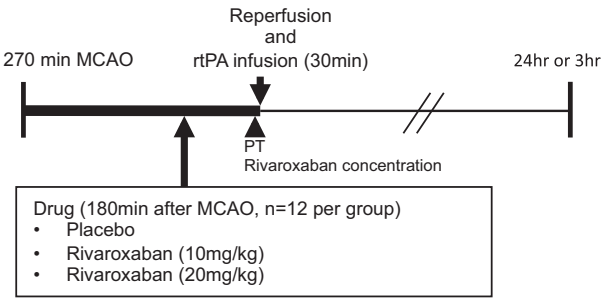

Figure 1. *Experimental protocol of filament middle cerebral artery occlusion (MCAO) with recombinant tissue plasminogen activator (rtPA) and rivaroxaban administration.*

Briefly, the left common and external carotid artery were exposed and ligated using 6-0 silk sutures. A 4-0 nylon suture coated with silicone rubber was inserted into the left internal carotid artery, and advanced gently for approximately 18 mm from the carotid bifurcation to occlude the middle cerebral artery. Regional cerebral blood flow in the territory of the left MCA was monitored before and during MCAO, and 30 minutes after reperfusion using laser Doppler flowmetry (ALF 21; ADVANCE, Tokyo, Japan). Following the operation, all animals were provided with regular drinking water ad libitum. Animals were sacrificed at the indicated time points after assessment of their neurological function with a 6-point score, as described by Bederson et al with minor modifications. (0 = no deficit, 1 = failure to extend right forepaw, 2 = circling to the contralateral side, 3 = falling to the right, 4 = unable to move spontaneously, 5 = dead).[11]

Rivaroxaban and rtPA Administration

Rivaroxaban (Bayer HealthCare AG, Wuppertal, Germany) was dissolved in a vehicle comprised of 60% polyethylene glycol (Wako, Osaka, Japan) and 40% distilled water. Rivaroxaban (10 mg/kg or 20 mg/kg) or placebo was administered to rats (n = 16 for each group) via a stomach tube 180 minutes after MCAO (ie, 90 minutes before reperfusion of MCAO and rtPA administration). The dose and time point were chosen because, in terms of the PT prolongation effect, preliminary examination showed that 20 mg/kg was the maximum dose and 90 minutes was the peak time point. rtPA (Alteplase10 mg/kg; Tanabe Mitsubishi Pharma, Osaka, Japan) was administered via a femoral vein catheter by continuous infusion over 30 minutes, which was initiated just before reperfusion after 270 minutes of MCAO.

Evaluation of Pharmacodynamics Effects

To evaluate the pharmacodynamic effects of rivaroxaban, we measured rivaroxaban plasma concentrations (n = 3) and PT (n = 5). Both were measured 90 minutes after drug administration (ie, just before rtPA administration). Four hundred fifty microliters of blood were mixed with .11 mol/L trisodium citrate in a 9:1 ratio, and plasma was obtained by centrifugation (15 minutes, 1500 g at 20° C). Rivaroxaban concentrations were measured using a high-performance liquid chromatography system coupled to a tandem mass spectrometer in selected reaction monitoring mode with a turbo ion spray (Shimadzu LC-10ADVP coupled with MS/MS API 4000; AB Sciex) as described previously.[12] For PT measurement, collected plasma samples were mixed with the thromboplastin reagent (Neoplastin Plus; Roche Diagnostics, Tokyo, Japan). PT was measured using a coagulometer (Coapresta-2000; Sekisui Medical, Tokyo, Japan) according to the manufacturer's instruction. For the normal control