GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 22, Number 1, 2018

Pp. 51–54
DOI: 10.1089/gtmb.2017.0152

CYP3A Activity and Rivaroxaban Serum Concentrations in Russian Patients with Deep Vein Thrombosis

Dmitriy Alexeyevich Sychev,[1] Arshak Vardanyan,[1] Aleksandr Rozhkov,[2] Edita Hachatryan,[2] Ani Badanyan,[1] Valery Smirnov,[2] Anna Ananichuk,[2] and Natalya Denisenko[1]

Background: Rivaroxaban is metabolized in the liver via CYP3A4, the cytochrome involved in the metabolism of nearly 50% of all medications. Thus, its effective concentration depends on multiple pharmacologic parameters.
Methods: The primary goal of our research was to study the correlation between the CYP3A family activity and the safety and efficacy of anticoagulant therapy with rivaroxaban in patients with deep vein thrombosis (DVT). Thirty one patients with DVT aged 21–83 years, 18 men and 13 women, received rivaroxaban (Xarelto) 30 mg/day for 21 days after diagnosis and 20 mg/day for the follow-up period of 6 months. During the study period, Doppler ultrasound was performed weekly to assess the clot dynamics and recanalization time.
Results: We found a direct statistically reliable correlation between CYP3A4 activity and both peak and trough rivaroxaban levels. A correlation was also found between the initial clot length and the time to full recanalization $r = 0.764$ (0.554–0.883), $p < 0.0001$. No significant link was found between either the glomerular filtration rate and peak rivaroxaban concentrations or between CYP3A4 activity and the treatment effectiveness parameters. No connection between renal function and rivaroxaban concentration was established in our study, which agrees with the clinical trials data that allow unlimited rivaroxaban use in patients with glomerular filtration rate >30 mL/min.
Conclusions: The direct link between the initial clot length and time to full recanalization that has been found means that patients with more advanced stages of thrombosis need more time to reach recanalization than their counterparts with a less severe condition.

Keywords: rivaroxaban, CYP3A, pharmacogenetics

Introduction

Rivaroxaban is one of the new oral anticoagulants that can be used to prevent embolism in deep vein thrombosis (DVT) and atrial fibrillation. Its effectiveness was originally proven as a thromboembolism prophylaxis agent in orthopedic surgery (Mueck *et al.*, 2008). So far, rivaroxaban was shown to be as effective as conventional therapy in clinical trials such as EINSTEIN DVT and EINSTEIN PE. Rivaroxaban's effectiveness as a stroke prevention measure was comparable with warfarin's in patients with valvular and nonvalvular atrial fibrillation (Patel *et al.*, 2011). One of the commonly mentioned disadvantages of rivaroxaban that limits its applications is no antidote that could be used in case of overdose. Recently, however, a new antidote for Xa factor inhibitors has been synthesized and its forthcoming introduction to clinical practice will raise the safety of Xa factor inhibitors (Connolly *et al.*, 2016). Still, when prescribing rivaroxaban, it is of vital importance to take certain pharmacokinetic concerns into account. Bioavailability and renal clearance of rivaroxaban depend on the activity of P-glycoprotein, which pumps the drug back into the intestinal lumen and the cells of the proximal tubule of the kidney. Rivaroxaban is metabolized in the liver through CYP3A4, the cytochrome involved in the metabolism of almost 50% of all medications, which, in turn, can induce or inhibit its activity. Concomitant therapy with CYP3A4 inhibitors may lead to increase in rivaroxaban serum concentration, causing adverse reactions such as bleeding. The opposite effect is seen when rivaroxaban is administered with CYP3A4-inducing agents, leading to a drop in rivaroxaban concentration and, therefore, decrease of its anticoagulant effect. It is also important to remember that CYP3A4 gene has single nucleotide polymorphisms that influence CYP3A4 activity and can alter the safety and efficacy of rivaroxaban in certain patients.

[1]Russian Medical Academy of Continuing Medical Education, Moscow, Russian Federation.
[2]I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation.

The primary goal of our research was to study the correlation between the CYP3A family activity and effectiveness of anticoagulant therapy in patients with DVT.

Materials and Methods

Thirty one patients with DVT were included in this study, aged 21–83 years, 18 men and 13 women. All patients took rivaroxaban (Xarelto) for 21 days after being diagnosed with DVT in doses of 15 mg twice a day (in the morning and at night), and then they were switched to 20 mg once a day in the morning dosing regimen. Exclusion criteria were taken from the EINSTEIN study, wherein they had successfully been used to study rivaroxaban efficacy, and were as follows: another indication for a vitamin K antagonist; creatinine clearance <30 mL per minute; clinically significant liver disease (e.g., acute hepatitis, chronic active hepatitis, or cirrhosis) or alanine aminotransferase level that was three times the upper limit of the normal range or higher; bacterial endocarditis; active bleeding or a high risk of bleeding, contraindications to anticoagulant treatment; systolic blood pressure >180 mm Hg or diastolic blood pressure >110 mm Hg; childbearing potential without proper contraceptive measures, pregnancy, or breastfeeding; concomitant use of strong cytochrome P-450 3A4 inhibitors (e.g., human immunodeficiency virus protease inhibitors or systemic ketoconazole) or inducers (e.g., rifampicin, carbamazepine, or phenytoin); participation in another experimental pharmacotherapeutic program within 30 days before screening; and a life expectancy of <3 months (Agnelli *et al.*, 2010). Patient characteristics are presented in Table 1.

Patients were followed up for 6 months. All patients underwent Doppler ultrasonography of the legs that helped establish the length of the clot on admission. During the study period, Doppler ultrasonography was performed weekly to assess the clot dynamics and recanalization time. Peak and trough rivaroxaban serum concentrations were measured in patients who have been taking 20 mg of the drug daily for no less than 2 weeks. Concentrations were measured using high-performance liquid chromatography mass spectrometric detection. CYP3A cytochrome family activity was evaluated based on urinary 6beta-hydroxycortisol to cortisol (6beta-OHF/F) ratio, the method used for CYP3A phenotyping (Furuta, 2003).

The normality of the sample distribution was evaluated using the Shapiro–Wilk test and taken into account in statistical method selection. The differences were considered as statistically significant at $p < 0.05$. To determine the correlation between quantitative characteristics, Spearman rank correlation coefficient (r) was calculated. Correlation coefficient (r) values from 0.3 to 0.7 ($p < 0.05$) indicated significant, but moderate positive correlation between the characteristics, whereas $r > 0.7$ ($p < 0.05$) indicated strong and significant correlation. All of the results have 95% confidence intervals. All data were distributed nonparametrically.

Table 1. Patient Characteristics

Number of patients	31
Mean age, years	39.8 ± 14.7
Male	18
Female	13
Mean glomerular filtration rate, mL/min	62.5 ± 24.6

Table 2. Results of CYP3A Activity and Clot Dynamics Evaluation

Pharmacokinetics	
Urinary 6beta-hydroxycortisol to cortisol (6beta-OHF/F) ratio	2.0 ± 2.9
Peak concentration, ng/mL	59.0 ± 89.3
Trough concentration, ng/mL	51.5 ± 84.2
Clot dynamics (based on Doppler ultrasonography measurements)	
Mean clot length on admission, cm	12.0 ± 7.2
Mean clot length 3 months after beginning of treatment, cm	1.1 ± 1.6
Mean change in clot length after 3 months treatment, cm	10.9 ± 6.0
Mean relative change in clot length after 3 months treatment, %	94.6 ± 6.9
Time to full recanalization, months	2.5 ± 1.3

Statistical analysis was performed using SPSS version 22.0 for Windows (SPSS, Inc., Chicago, IL). A two-side value <0.05 was considered statistically significant.

Results

Mean peak rivaroxaban concentration was 59.0 ± 89.3 ng/mL, trough level was 51.5 ± 84.2 ng/mL. 6beta-OHF/F ratio, the indicator of the CYP3A activity, was 2.0 ± 2.9.

Mean clot length on admission was 12.0 ± 7.2 cm. After 3 months of treatment, mean clot length has been reduced to 1.1 ± 1.6 cm ($p < 0.05$). Mean change in length was 10.9 ± 6.0 cm, which corresponds to 94.6% ± 6.9% relative change. Full recanalization was reached after 2.5 ± 1.3 months on average. All results are shown in Table 2.

There was a direct statistically reliable correlation between CYP3A activity and peak rivaroxaban level with $r = 0.695$ (0.443–0.845), $p < 0.0001$, as well as between CYP3A activity and trough rivaroxaban level with $r = 0.766$ (0.558–0.884), $p < 0.0001$. These correlations are graphically represented in Figures 1 and 2, respectively. A correlation was also found between the initial clot length and the time to full

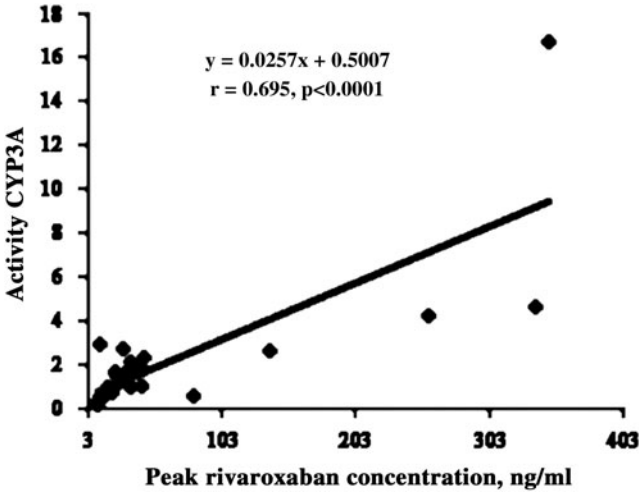

FIG. 1. Correlation between CYP3A activity and peak rivaroxaban concentration, ng/mL.

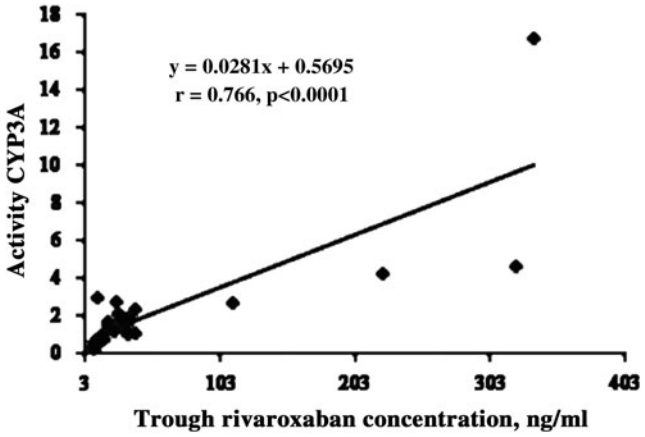

FIG. 2. Correlation between CYP3A activity and trough rivaroxaban concentration, ng/mL.

recanalization with $r=0.764$ (0.554–0.883), $p<0.0001$. No significant link was found between the glomerular filtration rate and peak rivaroxaban concentration $r=-0.101$ (–0.449 to 0.273), $p=0.558$ or trough rivaroxaban concentration $r=-0.269$ (–0.576 to 0.106), $p=0.144$.

Evaluation of CYP3A activity and treatment effectiveness parameters has yielded no significant correlation either: for the clot length change $r=0.108$ (–0.266 to 0.455), $p=0.561$ and for the time to full recanalization $r=-0.141$ (–0.481 to 0.235), $p=0.449$.

Discussion

CYP3A4 is involved in the metabolism of about 50% of all existing drugs and, therefore, drug interactions leading to its induction or inhibition, and the subsequent drug concentration changes are extremely common in clinical practice (Zhou *et al.*, 2007). Therefore, for CYP3A4 substrates such as rivaroxaban in our study, we need a method to evaluate its efficacy, as well as how it depends on the CYP3A4 activity level. It has long been considered that prothrombin time is unsuitable as a measure of the anticoagulant effect of rivaroxaban. In 2013, Samama *et al.* showed that rivaroxaban can increase prothrombin time in case of measurements performed with rivaroxaban-sensitive substances such as Neoplastin Plus® (Diagnostica Stago, Asnières-sur-Seine, France) or HemosIL RecombiPlasTin 2G (Instrumentation Laboratory, Bedford, MA). They have also noted that increase in rivaroxaban concentration does not necessarily increase bleeding risk (Samama *et al.*, 2013). This, again, stems from the fact that CYP3A4 is the key cytochrome in the metabolism of many other medications. In a study by Moore *et al.*(2014), researching a drug–drug–disease interaction in a combination of rivaroxaban with erythromycin, a mild CYP3A4 inhibitor, in patients with renal impairment, a slight increase of the peak rivaroxaban concentration and the area under the dose–response curve were observed, but no adverse reactions followed. Stöllberger and Finsterer (2017) have proved that concomitant rivaroxaban and carbamazepine use should be avoided due to carbamazepine being a P-glycoprotein and CYP3A4 inducer, which leads to faster rivaroxaban metabolism and weakens its anticoagulant effect. Another study by Hellwig and Gulseth (2013). has shown that a strong inhibitor such as ketoconazole does not influence the area under the dose–response curve, but increases the peak concentration by 70%. Mueck *et al.* (2013) have published data according to which it is safe to use rivaroxaban with mild CYP3A4 inhibitors, but concomitant use with strong inhibitors such as azole antimycotics, apart from fluconazole, and HIV protease inhibitors. As of now, there are no similar studies evaluating the effectiveness of rivaroxaban as an anticoagulant in connection with CYP3A activity and peak and trough drug levels, even though rivaroxaban has become widely popular in clinical practice. In 2015, Robertson *et al.* performed a Cochrane review (including 27,945 patients) that has proved the effectiveness of X factor inhibitors in patients with DVT. Our research has shown that CYP3A4 activity has no influence on the effectiveness or results of treatment, but does affect peak and trough concentrations. The strong correlation between CYP3A4 activity and rivaroxaban concentration proves the major role CYP3A4 plays in rivaroxaban metabolism. Although one would expect the change in rivaroxaban serum level to influence the effectiveness of the treatment, we see no connection between rivaroxaban concentration and clot dynamics. These conclusions could be biased due to a small number of patients in the study as well as long periods in-between ultrasound scans to review clot dynamics. However, the strong levels of correlation indicate high potency of the study for such analysis. In the future, it is essential to assess the thrombolysis progress and rivaroxaban serum concentration daily. No connection between renal function and rivaroxaban concentration has been established either. This fact agrees with the clinical trials data according to which rivaroxaban can be used without limitations in patients with glomerular filtration rate >30 mL/min (Haas *et al.*, 2014). The direct link between the initial clot length and time to full recanalization means that patients with more advanced stages of thrombosis need more time to reach recanalization than their counterparts with less severe condition.

Conclusions

Change in CYP3A4 activity influences peak and trough rivaroxaban serum concentrations, but does not, however, influence the effectiveness of treatment.

Limitations

This study had a small number of patients, no CYP3A4 and P-glycoprotein genotyping was carried out, and periods between clot dynamics measurements were too long.

Acknowledgments

The authors are grateful to E.S. Il'ina, at the Russian Medical Academy of Postgraduate Education, Ministry of Health of the Russian Federation, for assistance in the article preparation. This work was supported by the Russian Science Foundation (project 16-15-00227: ''Fundamental research and exploratory research in priority areas of research.'')

Compliance with Ethical Standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the

1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Author Disclosure Statement

The authors have indicated that they have no conflicts of interest with regard to the content of this article.

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Address correspondence to:
Alexandr Rozhkov, MD
I.M. Sechenov First Moscow State Medical University
Moscow 119435
Russian Federation

E-mail: rozkovsaha@mail.ru