THE POSSIBLE ROLE OF PHARMACOGENETICS WHEN RESUMING ANTICOAGULATION FOLLOWING INTRACRANIAL HEMORRHAGE

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Abstract: Intracranial hemorrhage (ICH) is the most feared complication of anticoagulation, therefore resuming anticoagulation following ICH is challenging. We present the case of a 76-year-old man with progression of left leg deep venous thrombosis (DVT). His previous anticoagulant DVT treatment was complicated by ICH and the anticoagulant was stopped. Without anticoagulant therapy, DVT symptoms worsened. Careful anticoagulant reintroduction was initiated. After two months of subcutaneous enoxaparin application, the patient desired a direct oral anticoagulant (DOAC). Several considerations are important prior to DOACs introduction: age, renal and hepatic function, drug-drug interactions, and bleeding risk. To avoid possible genetically determined DOAC pharmacokinetics alteration and drug-drug interactions, a pharmacogenetic analysis was performed. Following the pharmacogenetic findings, reduced dabigatran doses were introduced. Optimal plasma dabigatran concentrations were confirmed, and the further clinical course was uneventful. An individual approach with pharmacogenetic testing can provide additional information in selecting the appropriate medication in an optimal dosage.

Keywords: cytochrome P450, dabigatran, DOAC, P-glycoprotein, pharmacogenetic, thrombosis

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INTRODUCTION

Venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), is a major cause of morbidity and mortality worldwide. The estimation of VTE incidence is 1/1 000 persons annually with PE occurring in up to one third of the cases (1). Direct oral anticoagulants (DOACs) are recommended as the first-choice anticoagulant therapy for acute VTE treatment (2). Current guidelines highlight concomitant drug and comorbidity pharmacokinetic (PK) effects with DOACs (3). All DOACs are transmembrane substrates of the permeability glycoprotein (P-gp), an efflux transporter, which mediates drug export into the cells of the small intestine, blood-brain barrier, hepatocytes, and proximal tubule of the kidney. Consequently, intestinal absorption as well as biliary and urinary excretion can be altered by P-gp inhibition or induction (4-6). Pharmacogenomics of DOACs is a relatively new field that may enable optimal individual choice of the proper medication at the proper dose (4-6). Polymorphisms of genes encoding P-gp and the cytochrome P450 enzyme (CYP) might explain the interindividual variability of DOAC plasma concentrations and assist in predicting treatment failure or toxicity. Other than CYP450, human carboxylesterase gene polymorphisms (CES1) may also influence dabigatran and edoxaban pharmacokinetics (6).

Table 1. *Patient's pharmacogenetic profile*

Gene – allele	Genotype	Phenotype	Method
*CYP2C9 *2, *3*	*1/***2**	Intermediate metabolism	A
*CYP2C19 *2, *17*	*1/***17**	Rapid metabolism	A
*CYP3A4 *22*	*1/***22**	Intermediate metabolism	B
MDR1 (ABCB1) 1236C>T *MDR1 (ABCB1) 3435C>T*	C/**T** C/**T**	Intermediate transport activity	A
ABCG2 421C>A	C/C	Normal transport activity	A
UGT2B7 -161C>T	C/C	Normal metabolism	A

Variant alleles are bolded.
A – Real time PCR TaqMan® SNP Genotyping (Thermo Fisher Scientific, Foster City, CA, USA); B – Real time PCR TaqMan® DME Genotyping (Thermo Fisher Scientific, Foster City, CA, USA); PCR – polymerase chain reaction; SNP – single nucleotide polymorphism; DME – drug metabolizing enzyme

Table 2. *Patient's pharmacogenetic profile and drug roles in metabolic pathways*

Patient's genotype	Patient's phenotype	Drugs - Substrates	Drugs - Inducers
CYP2C9	**IM**	diazepam, phenobarbital	phenobarbital
CYP2C19	**RM**	diazepam, metoprolol, phenobarbital	phenobarbital
CYP3A4	**IM**	apixaban, diazepam, rivaroxaban	phenobarbital
UGT2B7	NM	dabigatran, diazepam	phenobarbital
MDR1 (ABCB1)	**ITA**	apixaban, dabigatran, diazepam, metoprolol, phenobarbital rivaroxaban	phenobarbital
ABCG2 (BCRP)	NTA	apixaban, rivaroxaban	

Risk findings are bolded. DOACs are underlined.
Abbreviations: IM – intermediate metabolism, RM – rapid metabolism, NM – normal metabolism, NTA – normal transport activity, ITA – intermediate transport activity; DOAC – direct oral anticoagulant

CASE REPORT

A 76-year-old man was referred to our Department for a second opinion due to DVT progression. Four months earlier, the patient underwent surgical removal of a meningioma. One month after the surgery he developed left leg proximal DVT. Subcutaneous (SC) dalteparin (7500 international units twice daily) was initiated, but the in-hospital course was complicated by intracranial hemorrhage (ICH). The anticoagulant was stopped, and an inferior vena cava (IVC) filter was implanted to prevent PE. The patient was discharged with the following daily therapy: acetylsalicylic acid (ASA) 100 mg, metoprolol 50 mg, phenobarbital 200 mg, ramipril 1.25 mg, and diazepam 5 mg. Anticoagulation was not resumed and IVC filter extraction was not considered. Within the next two months, the patient recovered neurologically, but pain and swelling of the left leg worsened due to thrombus progression (confirmed by ultrasound).

Table 3. *Coagulation parameters and dabigatran concentrations*

Coagulation tests	Basal results before dabigatran introduction	One week after dabigatran introduction (dose 110 mg BID)		One year after dabigatran introduction (dose 110 mg BID)	
		C trough	C peak	C trough	C trough
PT (%)	95	94	59	82	58
aPTT (s)	28.6	33.3	46.4	35.6	50.6
TT (s)	17.9	137.4	> 150	142.9	> 150
Fibrinogen (g/L)	2.7	3.1	2.8	3.9	4.3
DPC* (ng/mL)		66.2	175.5	65.3	190.1
Expected DPC (dose 150 mg BID) in patients treated with PE/VTE (ng/mL)		60 (39-95)	175 (117-275)	60 (39-95)	175 (117-275)

Abbreviations: BID – lat. bis in die, twice daily; aPTT – Activated partial thromboplastin time; C – concentration; DPC – dabigatran plasma concentrations; PE – pulmonary embolism; PT – prothrombin time; TT – thrombin time; VTE – venous thromboembolism
Coagulation assays were performed in fresh plasma samples within 4 hours of blood collection. PT using Innovin, aPTT using Actin FS, fibrinogen using a modified Clauss method with Multifibren U reagent, and TT using BC Thrombin Reagent on BCS XP analyzer (Siemens Healthcare Diagnostics, Marburg, Germany), whereas D-dimer was measured by the VIDAS D-Dimer Exclusion II assay on the mini VIDAS Immunoassay system (bioMérieux, Marcy l'Etoile, France).
*DPC was measured using in-house diluted thrombin time (dTT) with BC Thrombin as reagent applicated on the BCS XP analyzer (Siemens Healthcare Diagnostics, Marburg, Germany) and calibrated using STA-Dabigatran calibrators (Diagnostica Stago, Asnières sur Seine Cedex, France). Peak and trough samples at steady state were collected for DPC measurement.[7] C trough – plasma samples collected before the next dabigatran dose (within 10 – 16 h after last dabigatran dose); C peak – plasma samples collected 3 h after dabigatran administration.

Upon admission to our Department, the patient presented with severe, painful edema of the left leg, while the remaining physical status was within normal parameters, including liver and renal function. Several concerns arose regarding further management and were discussed by a multidisciplinary team. Anticoagulation resumption could stop thrombosis progression and leg symptoms; however, the patient had a high bleeding risk. Additionally, IVC filters are effective in PE protection, but may also provoke IVC thrombosis as well as other complications. As no further absolute contraindication for anticoagulation existed, mid-level enoxaparin dosage (0.4 mL subcutaneously twice daily) was initiated and IVC filter extraction ensued. ASA was stopped prior to enoxaparin introduction. Three months after admission, the patient requested to be treated with a DOAC. To avoid possible genetically determined DOAC pharmacokinetics alteration and drug-drug interactions (DDIs) with concomitant therapy, a pharmacogenetic analysis was performed (Table 1). Pharmacogenetic results indicated, among other findings, that the patient was a CYP3A4 intermediate metabolizer.

When we compared the metabolic pathways for all concomitant medicines with DOACs to the patient's pharmacogenetic profile (Table 2), three of them, in addition to DOACs, were P-gp substrates. Such a high load of P-gp can cause slower efflux transport function and possible prolonged bioavailability of P-gp drug substrates, leading to their higher concentrations.

It should be emphasized that phenobarbital is both a P-gp substrate and an inductor which, in this array, might have a positive influence on efflux function. As rivaroxaban, apixaban, and edoxaban are metabolized via hepatic CYP, primarily CYP3A, dabigatran was chosen for therapy as it has, among other available DOACs, the most favorable pharmacogenetic profile with the least number of risk alleles.

Considering the pharmacogenetic profile and possible DDI, slightly reduced doses of dabigatran 110 mg twice daily were introduced, along with monitoring of coagulation parameters and dabigatran plasma concentration (DPC). Peak and trough plasma samples at steady state

were collected for determination of the drug concentration, and optimal DPC was confirmed (Table 3).

In a three-month follow-up visit, duplex-ultrasound confirmed leg edema regression and significant recanalization of deep veins with normal findings of D-dimer concentration (0.12 mg/L fibrin equivalent units, FEU) as well as other coagulation parameters, including DPC, without bleeding complications. At the next three-month follow-up visit the patient complained of intermittent palpitations, and 24-hour electrocardiogram confirmed paroxysmal atrial fibrillation indicating the need for lifelong anticoagulation (3). Dabigatran was continued, and the further clinical course was uneventful. One year after dabigatran initiation, duplex-ultrasound confirmed deep vein recanalization with normal findings of D-dimer concentration (0.18 mg/L FEU) as well as other coagulation parameters, including DPC (Table 3), without bleeding complications.

DISCUSSION

Our patient required detailed considerations regarding the administration of anticoagulant therapy due to a combination of interfering factors (recurrent DVT, previous ICH during anticoagulation, older age, and concomitant medication). ICH is one of the most devastating potential complications of anticoagulation, therefore anticoagulation reintroduction following ICH requires careful balancing between drug efficacy and safety. Even though DOACs are often referred to as a uniform drug class, there is increasing evidence from indirect comparisons and observational studies that each DOAC has its own specific risk profile (8, 9). Furthermore, physicians should be aware of potential DDIs with DOACs. Drugs and other P-gp and/or CYP3A4 inductors may decrease DOAC plasma concentrations leading to an increased risk for thromboembolic events, while P-gp and/or CYP3A4 inhibitors may increase DOAC concentrations leading to an increased bleeding tendency. Pharmacokinetic DDIs that may occur in association with DOACs are largely mediated by the P-gp efflux transporter protein alone (all DOACs) or in combination with CYP enzymes (except for dabigatran) (6, 8, 10, 11). Since current guidelines do not recommend one DOAC over another, we performed a preemptive pharmacogenetics analysis in order to determine which product would induce a better clinical response while avoiding adverse events (12). The pharmacogenetic profile should include genes for CYP3A4, CYP3A5, CYP2J2, and CYP1A2 enzymes, as well as transporters P-gp, ABCG2, and SLCO1B1, that is, all major enzymes and transporters involved in DOAC metabolism to enable a more optimal individual choice among DOACs (6).

Although DOACs pharmacogenetic studies have not yet yielded unambiguous results and have not been translated into some form of recommendations, we believe that the approach described may help clinicians. We intend to confirm this claim in an ongoing clinical study.

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SAŽETAK

PONOVNO UVOĐENJE ANTIKOAGULANTE TERAPIJE NAKON INTRAKRANIJSKOG KRVARENJA – MOGUĆA ULOGA FARMAKOGENETIKE

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Intrakranijsko krvarenje (ICH, od eng. intracranial hemorrhage) jedna je od najopasnijih komplikacija antikoagulantne terapije. Potreba za nastavkom antikoagulantne terapije nakon ICH-a je izazovno pitanje u praksi. U radu smo prikazali slučaj 76-godišnjeg bolesnika s progresijom duboke venske tromboze lijeve noge. Njegovo prethodno liječenje antikoagulatnom terapijom zbog duboke venske tromboze kompliciralo se intrakranijskim krvarenjem i antikoagulantna terapija je prekinuta. Bez antikoagulantne terapije simptomi duboke venske tromboze su se pogoršavali. Započeto je pažljivo ponovno uvođenje antikoagulantnog lijeka. Nakon dva mjeseca potkožne primjene enoksaparina bolesnik je želio nastaviti liječenje direktnim oralnim antikoagulantom (DOAK). Prije uvođenja DOAK-a važno je razmotriti nekoliko čimbenika: dob, bubrežnu i jetrenu funkciju, interakcije lijekova te rizik krvarenja. Kako bi se izbjegle moguće genetički određene promjene farmakokinetike DOAK-a i interakcije lijek-lijek, napravljena je farmakogenetička analiza. Sukladno farmakogenetičkom nalazu uveden je dabigatran u smanjenoj dozi. Potvrđene su optimalne koncentracije dabigatrana u plazmi, a daljnji klinički tijek bio je bez komplikacija. Individualan pristup s farmakogenetičkim ispitivanjem može pružiti dodatne informacije u odabiru odgovarajućeg lijeka u optimalnoj dozi.

Ključne riječi: citokrom P450, dabigatran, DOAK, P-glikoprotein, farmakogenetika, tromboza

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