International Journal of Cardiology 293 (2019) 73–75

Contents lists available at ScienceDirect

International Journal of Cardiology

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

Removal of dabigatran using sorbent hemadsorption☆

Alexandra A. Angheloiu [a], George O. Angheloiu [b,c,*]

[a] Temple University, Philadelphia, PA, USA
[b] University of Pittsburgh Medical Center, Williamsport, PA, USA
[c] The Heart Doctor, Manheim, PA 17545, USA

ARTICLE INFO

Article history:
Received 9 June 2019
Accepted 27 June 2019
Available online 29 June 2019

Keywords:
Dabigatran
Removal
Sorbent
Hemadsorption
Adsorption
aPTT

ABSTRACT

Background: The Redual PCI trial has demonstrated the safety of dabigatran and ticagrelor or clopidogrel combination in preventing strokes in patients with atrial fibrillation. There was 15.4% risk of hemorrhage in the dabigatran/ticagrelor or clopidrogel arm, lower than that of triple therapy with warfarin, aspirin and ticagrelor or clopidogrel. While idarucizumab is an effective antidote for dabigatran, there is no good method for antagonizing both dabigatran and ticagrelor. We tested in this study a hemadsorbtion method for removing dabigatran that we had previously successfully applied in the removal of ticagrelor from human blood.

Methods: 100 mL 4% BSA solution pre-incubated with dabigatran was passed through 10, 20 and 40 mL sorbent columns and dabigatran concentration was measured from the affluent and effluent solution using LC-MS/MS. For testing the effect of dabigatran removal on the aPTT value one human volunteer was administered oral dabigatran etexilate mesilate 150 mg. Plasma was collected 4 h after dabigatran administration and then in three experiments 20 mL of collected plasma was circulated through three different 10 mL CytoSorb columns over a duration of 5 min. aPTT was measured from plasma at baseline prior to drug administration, then post blood collection (mixed plasma) and from the adsorbed plasma as well.

Results: Dabigatran concentration, as measured by LC-MS/MS, decreased from 1456 ± 331 nM (greater than the therapeutic level of 743 nM) to 67 ± 59 nM ($P = 0.002$) with the 10 mL CytoSorb column, while with the 40 mL column it dropped to undetectable levels. In one human volunteer experiment the aPTT was on average 29.2 ± 0.4 in the 3 baseline samples, 34.7 ± 1.8 s after oral dabigatran (mixed plasma), and 25 ± 0.7 s after plasma was passed through CytoSorb (adsorbed plasma) ($P = 0.000025$ and 0.0000002 for comparison between baseline plasma and mixed plasma, as well as the dabigatran mixed plasma and post-adsorption values respectively).

Conclusion: Dabigatran is robustly removed by a sorbent hemadsorption method already proven successful for the P2Y12 receptor antagonist ticagrelor. Dabigatran removal restores the aPTT to below baseline values, suggesting that sorbent hemadsorption could clinically reverse the anticoagulant effect of this drug.

1. Introduction

The recent Redual PCI trial has demonstrated the safety of dabigatran and ticagrelor or clopidogrel combination in preventing strokes in patients with atrial fibrillation [1]. There was a 15.4% risk of hemorrhage in the dabigatran/ticagrelor or clopidrogel arm, lower than that of triple therapy with warfarin, aspirin and ticagrelor or clopidogrel.

While an antidote (idarucizumab) has been developed for dabigatran as a reversal agent, there is no clinical antidote for ticagrelor or clopidogrel. Our group has recently reported a successful method for removing ticagrelor [2] and edoxaban [3]. The same removal method was applied in this paper.

A clinical laboratory measure of the anticoagulant properties of dabigatran is offered by a simple aPTT measurement [4]. We hypothesize that dabigatran can be removed by sorbent beads from albumin solution and that dabigatran removal restores the coagulation properties of plasma as expressed by the aPTT in a human subject.

2. Methods

CytoSorb (donated by Cytosorbents, Monmouth Junction NJ) is a styrene copolymer with bead diameter of 425–1000 μm and surface area of 850 m^2/g.

2.1. Definitions

Dabigatran removal is a value expressed in percentages and equal with the ratio (affluent concentration – effluent concentration)/(affluent concentration) where the affluent and effluent concentration (is) the dabigatran concentration entering and respectively exiting the sorbent column. Initial, mixed, and adsorbed BSA solution refers to the vehicle

Abbreviations: LC-MS/MS, liquid chromatography with tandem mass spectrometric detection; BSA, bovine serum albumin; aPTT, activated partial thromboplastin time.

☆ A grant was awarded by Boehringer Ingelheim towards completion of this study.

* Corresponding author.
E-mail address: angheloiug@upmc.edu (G.O. Angheloiu).

https://doi.org/10.1016/j.ijcard.2019.06.078
0167-5273/

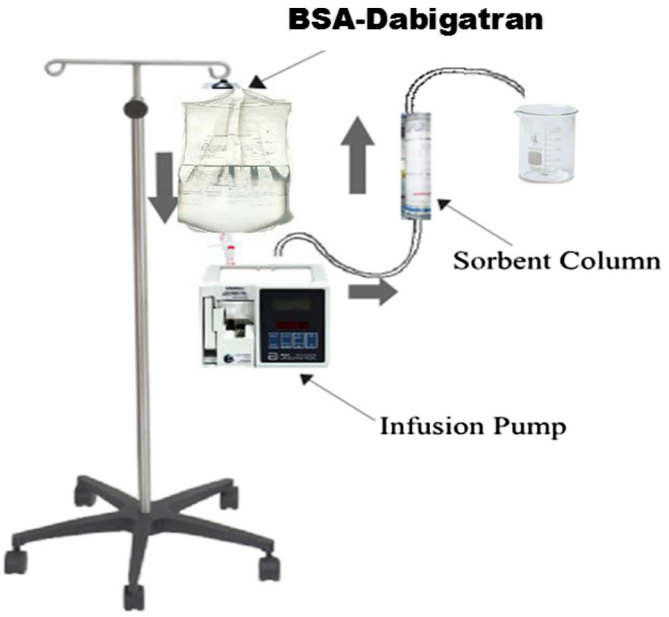

Fig. 1. First-pass dabigatran removal experimental set-up illustrating a CytoSorb column and the pump used to circulate the drug-vehicle solution.

solution before being respectively pre-incubated with the drug, after mixing, and after being adsorbed through the sorbent column at various intervals (of) time. The filtration velocity expressed as output in mL/min represents the velocity of the fluid vehicle (BSA solution) carrying the drug dabigatran and being driven by the perfusion pump and circulated through the circuit from Fig. 1.

Dabigatran-BSA solution was prepared by incubating the mix for approximately 30 min prior to use at room temperature.

The BSA-dabigatran experiments were performed in a first-pass manner (Fig. 1). (100) mL of BSA-dabigatran solution mix were housed in a 1 L perfusion bag and pushed with a velocity of 1 mL/min by a Lifecare 5000 Infusion System pump (Abbott, Green Oaks IL) through sorbent columns of respectively 10 mL, 20 mL and 40 mL capacity after careful deaeration and priming of the tubing lines. The drug vehicle was transferred through the sorbent column in an anti-gravitational manner [2]. Each BSA experiment lasted 100 min. The resulting adsorbed solution was collected in jars from which samples were taken for drug measurement assays at the end of each first-pass experiment. The experiment ran through the 10 mL columns were performed successively both with 4% and 0.4% BSA solutions in order to assess the influence of the BSA concentration on the removal process. Experiments using the 10 mL columns were performed in 3 separate runs, while all remaining experiments were completed in single runs.

Assay. Drug concentrations were measured in the mixed and final adsorbed drug-solution mix, at the end of each 100 min experiment. Dabigatran concentrations were measured using LC-MS/MS assay as detailed elsewhere at the Boehringer Ingelheim Pharmaceuticals laboratories in Ridgefield, CT [5].

To test the effect of dabigatran removal on the plasma aPTT, one subject, a healthy volunteer (author GOA), was administered dabigatran etexilate mesylate 150 mg orally, after local IRB approval. A consent form was signed. Plasma was separated (5 min, 5600 RPM) from blood collected in 2.7 mL 0.109 M sodium citrate vacutainers (Becton Dickinson, Franklin Lakes, NJ) 4 h after dabigatran administration. Then in three experiments 20 mL of collected plasma was circulated through three different 10 mL CytoSorb columns over a duration of 5 min in an anti-gravitational manner. aPTT was measured from the baseline plasma prior to drug administration, plasma post blood collection, adsorbed plasma, along with aPTT from 1 mL + 1 mL mix of baseline and adsorbed plasma. The aPTT was measured twice for each sample and the average was used in calculations [4].

2.2. Statistics

Student's *t*-test for paired sets was used for multiple numerical variables analysis (Open Office 3.1, The Apache Software Foundation, Los Angeles, CA). The numerical variables compared were specifically the drug concentrations in the affluent and effluent vehicle solutions, as well as the aPTT values in the 4 plasma sets tested.

3. Results

Dabigatran was removed in a first-pass experiment extremely well by CytoSorb with maximum >99% removal (Fig. 2). Best removal rate was achieved with the 40 mL CytoSorb columns.

The removal efficiency was substantial even with lower CytoSorb column volumes. The 10 mL CytoSorb columns' experiment performed in 4% BSA succeeded to drop the dabigatran levels from an average of 1456 nM ± 331 nM (higher than the clinical therapeutic level of 743 nM) to 67 nM ± 59 nM ($P = 0.002$) in a 3-run experimental set, for an average removal rate of 94%. The 20 mL CytoSorb column performed slightly better than the same value of 94% (Fig. 2). A complete removal was achieved with the 40 mL CytoSorb column from 1074 nM dabigatran to undetectable levels, below the dabigatran IC50 for thrombin inhibition of 9.2 nM [5].

3.1. Coagulation reversal effect of dabigatran removal

Administration of oral dabigatran caused as expected a rise in the aPTT from a baseline of 29.2 ± 0.4 to 34.7 ± 1.8 s after administration ($P = 0.000025$). Sorbent removal of dabigatran in four 3-sample series of experiments reduced the aPTT values from 34.7 ± 1.8 in the mixed plasma collected after administration s to sub-normal levels of 25 ± 0.7 s in the adsorbed samples ($P = 0.0006$ and 0.00064 for comparison with the dabigatran mixed plasma and baseline plasma). The average aPTT values of an equal quantity combination of adsorbed plasma and

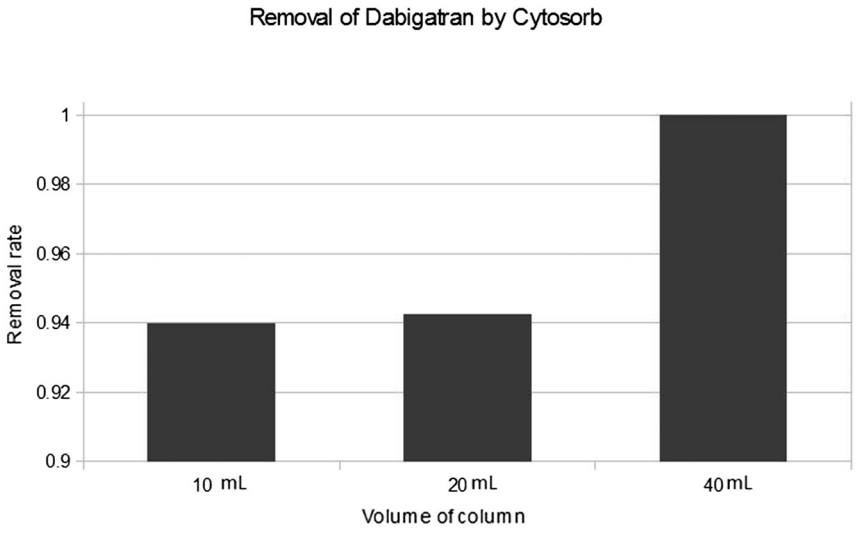

Fig. 2. Removal of dabigatran using CytoSorb during first-pass experiments using three types of CytoSorb columns of 10 mL, 20 mL and 40 mL volume respectively.

baseline plasma were intermediary between the adsorbed values and baseline (27.4 ± 0.9 s, $P = 0.02$ for comparison with baseline).

3.2. Effect of BSA concentration

The experiments using 10 mL columns were performed both using 4% as well as 0.4% BSA in three separate runs for each albumin dilution. The removal efficiency was the same with the two albumin dilutions: 94% ($P = 0.5$).

4. Discussion

4.1. Removal efficiency

We succeeded to clear dabigatran using CytoSorb from BSA solution with high efficiency. As expected adsorption was seemingly better using higher masses of sorbent, with higher doses of sorbent removing more drug (Fig. 2).

Varying the albumin concentration did not change the dabigatran removal efficiency, suggesting that albumin does not interfere with the dabigatran removal process.

4.2. Clinical significance

Our method was able to reach >99% dabigatran removal in BSA solution in a 100 min first-pass experiment. Since the drug is approximately 35% bound to plasma proteins [6], our results in albumin solution suggest that the drug could be removed from plasma proteins. Thrombin is a plasma protein, and the interaction between dabigatran and thrombin is of a reversible nature, hence one may imagine that sorbents may be able to remove dabigatran from thrombin.

In removal experiments with another reversible agents, this time acting at the level of the $P2Y_{12}$ platelet receptors (ticagrelor) or factor Xa, CytoSorb achieved a removal rate of approximately 99%–100% in BSA solution as well as >99% in human blood experiments [2,3]. This could suggest that sorbents could be successful in removing dabigatran from human blood as well.

At therapeutic mean concentrations (350 ng/mL or 743 nM) the total amount of dabigatran in the human body is 21 mg for a distribution volume of 60 L. We were able to remove completely approximately 0.35 mg of dabigatran using a 40 mL CytoSorb column. Hence a total amount of 2400 mL CytoSorb may eventually be needed to remove completely 21 mg of drug in a clinical experiment.

4.3. Coagulation reversal effect of dabigatran removal

Administration of oral dabigatran caused as expected a rise in the aPTT values in a healthy volunteer that was administered the drug ($P = 0.004$). Sorbent removal of dabigatran from plasma collected from the volunteer at the time of peak concentration reduced the aPTT values from 34.7 ± 1.8 s (at peak concentration) to sub-normal levels of 25 ± 0.75 s after adsorption ($P = 0.0000002$).This could suggest that sorbent removal of dabigatran in future in-vivo experiments may revert the coagulant effect of dabigatran.

We suspect that the sorbent-treatment of the collected plasma decreased the aPTT to subnormal levels (25 ± 0.75 s versus 29.2 ± 0.1 at baseline, $P = 0.0000001$) through removal of protein moieties mitigating the coagulation and fibrinolytic cascade. A proof towards this theory was the fact that mixing the adsorbed plasma with baseline normal plasma increased the aPTT from 25 ± 0.75 s to 27.4 ± 0.9 ($P = 0.0035$). A less aggressive sorbent-treatment of dabigataran mixed plasma should be able to address this problem, by increasing the filtration velocity or decreasing the amount of sorbent used.

There are three clinical scenarios where sorbent removal of dabigatran could be important. Cardiac patients are frequently on both antiplatelet and anticoagulant agents, such as in the Redual PCI study [1]. Since our method can remove dabigatran, the P2Y12 antagonist ticagrelor and factor Xa antagonist edoxaban [2,3], the hemadsorption route would be the most suitable in case of a hemorrhage caused by this drug combination. A second scenario is the possibility of removing the drug prior and during open heart surgery. Usually the blood is circulated through a cardiopulmonary bypass machine, which would allow one to pass the former through a sorbent column. In a third but similar scenario, of vascular surgery, the blood is bypassed through a shunt in order to avoid bleeding in the area served by the artery operated on. The shunted blood can be directed to a sorbent column before being sent back to the patient.

A plus of our procedure is the possibility of removing other types of cardiac active medication with significant side effects, including ticagrelor, edoxaban and rivaroxaban (factors Xa inhibitor) or radiocontrast agents, as demonstrated by our group and others in several in-vitro and in-vivo studies [2,3,7–9].

5. Conclusion

Dabigatran is vigorously removed from BSA solution during bench hemadsorption experiments and its removal normalizes the aPTT values in a subject that was administered dabigatran prior to plasma collection, creating the premises of a possible all-inclusive method of dabigatran, factor Xa antagonists and ticagrelor removal.

This study was funded by Boehringer-Ingelheim.

Acknowledgement

We kindly acknowledge the laboratory personnel at UPMC Susquehanna for their precious technical assistance.

Declaration of Competing Interest

No conflicts of interest for George O. Angheloiu and Alexandra A. Angheloiu.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.06.078.

References

[1] C.P. Cannon, D.L. Bhatt, J. Oldgren, et al., Dual antithrombotic therapy with dabigatran after PCI in atrial fibrillation, N. Engl. J. Med. 377 (2017) 1513–1524.
[2] G.O. Angheloiu, B. Gugiu, C. Ruse, et al., Ticagrelor removal from human blood, JACC, Basic to Translational Science 2 (2017) 135–145.
[3] A.A. Angheloiu, Y. Tan, C. Ruse, S.A. Shaffer, G.O. Angheloiu. In-vitro sorbent-mediated removal of edoxaban from human plasma and albumin solution. (Submitted).
[4] D. Shimomura, Y. Nakagawa, H. Kondo, T. Tamura, M. Amano, Y. Hayama, N. Onishi, Y. Tamaki, M. Miyake, K. Kaitani, C. Izumi, M. Hayashida, A. Fukuda, F. Nakamura, S. Kawano, Relationship between plasma dabigatran concentration and activated partial thromboplastin time in Japanese patients with non-valvular atrial fibrillation, J Arrhythm. 31 (4) (2015 Aug) 183–188.
[5] J. Antovic, M. Skeppholm, J. Eintrei, et al., Evaluation of coagulation assays versus LC-MS/MS for determinations of dabigatran concentrations in plasma, Eur. J. Clin. Pharmacol. 69 (2013) 1875–1881.
[6] W. Eisert, N. Hauel, J. Stangier, et al., Dabigatran: an oral novel potent reversible nonpeptide inhibitor of thrombin, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 1885–1889.
[7] G.O. Angheloiu, H. Hänscheid, C. Reiners, W.D. Anderson, J.A. Kellum, In vitro catheter and sorbent-based method for clearance of radiocontrast material during cerebral interventions, Cardiovasc Revasc Med 14 (2012) 207–212.
[8] G.O. Angheloiu, H. Hänscheid, X. Wen, V. Capponi, W.D. Anderson, J.A. Kellum, Experimental first-pass method for testing and comparing sorbent polymers used in the clearance of iodine contrast materials, Blood Purif. 34 (2012) 34–39.
[9] K. Hassan, J. Kannmacher, P. Wohlmuth, U. Budde, M. Schmoeckel, S. Geidel, Cytosorb adsorption during emergency cardiac operations in patients at high risk of bleeding, Ann. Thorac. Surg. 1 (2019) 45–51.