European Journal of Obstetrics & Gynecology and Reproductive Biology 210 (2017) 139–143

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

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

Full length article

Post-pregnancy aspirin resistance appears not to be related with recurrent hypertensive disorders of pregnancy

Carolien N.H. Abheiden[a,*], Wessel W. Fuijkschot[b], Arda Arduç[a], Jeske J.K. van Diemen[b], Ankie M. Harmsze[c], Marjon A. de Boer[a], Abel Thijs[b], Johanna I.P. de Vries[a]

[a] Department of Obstetrics and Gynecology, Institute for Cardiovascular Research (ICaR-VU), VU University Medical Center, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands
[b] Department of Internal Medicine, Institute for Cardiovascular Research (ICaR-VU), VU University Medical Center, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands
[c] Department of Clinical Pharmacology and Pharmacy, VU University Medical Center, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 13 September 2016
Received in revised form 1 December 2016
Accepted 19 December 2016
Available online xxx

Keywords:
Aspirin
Aspirin resistance
Hypertensive disorders of pregnancy
Low-molecular-weight heparin
Preeclampsia

ABSTRACT

Objective: The FRUIT-RCT concluded that low-molecular-weight heparin added to aspirin compared to treatment with aspirin alone is beneficial in the prevention of early-onset hypertensive disorders of pregnancy (HD) in women with inheritable thrombophilia and prior HD and/or a small-for-gestational age (SGA) infant leading to delivery before 34 weeks gestation. The aim of this study is to answer the question whether aspirin resistance is associated with recurrent HD.

Study design: Women with and without recurrent HD matched for age, study arm, and chronic hypertension were invited for this follow-up study 6–16 years after they participated in the FRUIT-RCT. Aspirin resistance was tested after 10 days of aspirin intake using three complementary tests: PFA-200, VerifyNow® and serum thromboxane B_2 (TXB_2). An independent *t*-test, Mann-Whitney *U* test, Fisher's Exact test and Chi^2 test were used for the statistical analyses.

Results: Thirteen of 24 women with recurrent HD and 16 of 24 women without recurrent HD participated. The prevalence of laboratory aspirin resistance was 34.5% according to the PFA-200, 3.4% according to the VerifyNow® and 24.1% according to TXB_2. The prevalence of aspirin resistance by any test was 51.7%. Aspirin resistance per individual test did not differ between women with and without recurrent HD. Aspirin resistance measured by any test occurred more frequently in women without recurrent HD ($p < 0.01$), irrespective of low-molecular-weight heparin.

Conclusions: No relation could be demonstrated between recurrent HD and aspirin resistance per test, measured up to 16 years after pregnancy. On the contrary, complementary aspirin resistance measurements were encountered more frequently in women without recurrent HD.

Introduction

After a recent trial, concluding that low-molecular-weight heparin when added to aspirin could potentially prevent early-onset hypertensive disorders of pregnancy (HD: preeclampsia, eclampsia and HELLP-syndrome) in women with inheritable thrombophilia and adverse obstetric history, a new research question arose [1]. In a letter to the editor Bujold et al. questioned whether the effect of low-molecular-weight heparin could have been mainly beneficial for the subgroup of women resistant to aspirin [2]. The term aspirin resistance is under debate, nowadays a term like aspirin non-responsiveness is also used.

About one third of cardiovascular high risk patients and trauma patients have been shown to be resistant to aspirin [3–8]. This is in line with the prevalence of aspirin resistance been found in women taking aspirin during pregnancy [9–11]. Two studies concluded that adjusting aspirin dosage might be needed to prevent HD in high risk women [9,10]. A third study concluded that aspirin resistance might be related to the occurrence of HD [11]. However, the relation between aspirin resistance and the occurrence or recurrence of HD has not been well explored. Elucidating this relation could lead to more individualized treatment in women who use aspirin during pregnancy and thereby improve their pregnancy outcomes. The aim of this study is to explore whether there is a relation between aspirin resistance and recurrent HD in

* Corresponding author.
E-mail address: c.abheiden@vumc.nl (C.N.H. Abheiden).

http://dx.doi.org/10.1016/j.ejogrb.2016.12.023
0301-2115/

the currently non-pregnant women who participated in the FRactionated heparin in women with Utero-placental Insufficiency and Thrombophilia randomized controlled trial (FRUIT-RCT) [1]?

Materials and methods

Participants

This study is a follow-up study of the FRUIT-RCT [1]. In the FRUIT-RCT, 139 women were included in the Netherlands (n = 126), Sweden (n = 3) and Australia (n = 10) between January 2000 and December 2009. All women had previous HD and/or a SGA-infant, delivered before 34 weeks gestation and had inheritable thrombophilia. Women were randomized to the combination of low-molecular-weight heparin and aspirin, or aspirin alone. For the current follow-up study, performed 6–16 years after women joined the FRUIT-RCT, we invited all participants from the Dutch subsample with HD during their study pregnancy (n = 24) and 24 matched for age, study arm and chronic hypertension without HD. Since the effect of aspirin resistance on the presence of HD is unknown, a power calculation could not be performed. Therefore, we invited all women with recurrent HD during the FRUIT-RCT to provide information for future studies. Exclusion criteria were: diabetes mellitus, use of drugs known to alter platelet function (e.g. NSAID's, beta-lactam antibiotics, SSRI's and amitriptyline, chronic use of antiplatelet agents) within two weeks before enrollment, major surgical procedure within one week before enrollment, a cardiovascular event within three months before enrollment and abnormal cell counts of hemoglobin, hematocrit, leucocytes and/or thrombocytes. The study was conducted in accordance with the Helsinki II Declaration and was approved by the Institutional Review Board of the VU University Medical Center in Amsterdam, the Netherlands. The study was registered at the Dutch Trial Register (Nederlands Trialregister; www.trialregister.nl) with number NTR5106. After written informed consent, we visited all women in their own local hospital.

Measurements

An update of the medical history after the FRUIT-RCT pregnancy, current use of medication and lifestyle habits was obtained via a questionnaire. Participants were instructed to take one tablet of aspirin (acetylsalicylic acid, 80 mg, non-enteric-coated) at 8pm for ten days. Venous blood samples were collected after an overnight fast at 8am. Blood samples were taken on two separate days; on day one, before usage of aspirin, and on day eleven, after ten days of aspirin intake. On day one, aspirin resistance was measured alongside with hemoglobin, hematocrit, thrombocytes and leucocyte counts. On day eleven two blood samples were collected within a timeframe of five minutes, to analyze aspirin resistance. The mean of these two results on day eleven was used in the analysis. Aspirin resistance was tested with three different tests: Platelet Function Analyzer-200 (PFA-200, INNOVANCE® PFA-200 System, Siemens Healthcare, Marburg, Germany); the VerifyNow® point-of-care system (Accumetrics, CA, USA); and serum thromboxane B_2 (TXB_2) level using an enzyme immunoassay kit (Assay Designs®, Ann Arbor, MI, USA).

PFA-200 measures the process of primary hemostasis [12]. For the analysis, citrated whole blood is passed through a capillary. The system measures platelet plug formation; the capillary will occlude. The time needed for complete obstruction of the capillary is the closure time (CT). A CT of $\leq$150 s was used for the dichotomous definition of aspirin resistance [9,10]. PFA has a theoretical maximum of 300 s which means that any CT >300 s is reported as 301 s. The Collagen/Epinephrine cartridge was used.

VerifyNow® utilizes arachidonic acid as an agonist to measure the antiplatelet effect of aspirin specifically along the pathway of inhibition of COX-1. A small tube of whole blood is inserted into an aspirin cartridge. The cartridges including the tube are inserted into VerifyNow® to measure the change in light transmittance through a patient's blood sample which results into Aspirin Reaction Units (ARU) [13]. An ARU of $\geq$550 was used for the dichotomous definition of aspirin resistance [14,15]. The aspirin cartridge was used.

Serum TXB_2 is a direct measure of the capacity of platelets to synthesize TXA_2 and a specific measure of the pharmacological effect of aspirin on platelets [16]. Directly after blood collection, blood samples were placed in a stove for one hour at 37° Celsius. After one hour, the blood samples were centrifuged for 10 min with 3000 rotations per minute. All serum samples were stored at −80° Celsius and analyzed within six months in the laboratory for hematology, unit thrombosis and hemostasis of the Radboud University Medical Center in Nijmegen, the Netherlands. A TXB_2 concentration above the highest quartile was defined as aspirin resistant [11].

Statistics

Baseline characteristics between the group with and without HD during the FRUIT-RCT were examined using an independent *t*-test, Mann-Whitney *U* test, or Chi^2 test. Results of different devices were given in both continuous and dichotomized outcomes. Differences in aspirin resistance between women with and without HD were tested with an independent *t*-test, Mann-Whitney *U* test, Fisher's Exact test, or Chi^2 test was used. Moreover, aspirin resistance occurrence was also examined taking into account the treatment arm during the FRUIT-RCT and examined between four groups: women with and without HD and with or without low-molecular-weight heparin treatment during the FRUIT-RCT using a Fisher's Exact test or Chi^2 test.

Statistical analyses were performed with IBM SPSS version 22.0 (SPSS Inc, Chicago, USA). A two-tailed p-value < 0.05 was considered to be significant.

Results

From the 48 women, two died (one from a cerebral lymphoma, one from a cerebral aneurysm), five were lost to follow-up and 12 refused participation. Twenty-nine participated, 13 with and 16 without recurrent HD. Four of the six women who had early-onset recurrent HD during the FRUIT participated. There was no difference in obstetrical history between de 29 participants and women who did not participate (data not shown).

There were no differences in baseline characteristics between the groups (Table 1).

We asked during the second visit whether all ten tablets of aspirin were taken and every participants confirmed this. The results of aspirin resistance in relation to recurrence of HD during FRUIT-RCT are presented in Table 2. The prevalence of aspirin resistance per device is depicted in Fig. 1.

Differences in aspirin resistance per device was not statistically significant between women with and without HD taking into account low-molecular-weight heparin usage during the FRUIT-RCT (p = 0.20 for PFA-200, p = 0.41 for VerifyNow®, p = 0.88 for TXB_2). Aspirin resistance measured by any test did differ (p = 0.04) between the four groups (Fig. 2).

Comment

Evaluating the results of this cohort of thrombophilic women, we did not demonstrate a relation between aspirin resistance and