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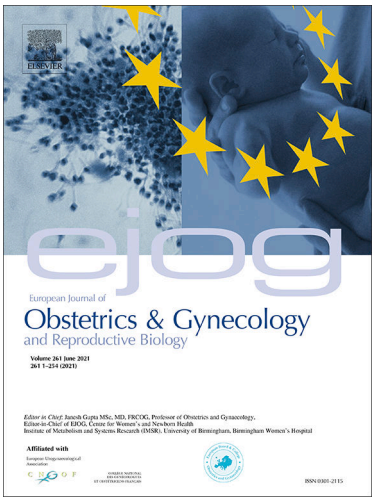

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The effect of low-dose aspirin on platelet function during pregnancy compared to placebo: an explorative study

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INTRODUCTION

Low-dose aspirin has been widely implemented for the prevention for preterm preeclampsia.(1, 2) It also has the potential to prevent fetal growth restriction, preterm birth and perinatal mortality.(3-8) The common underlying mechanism of these pregnancy complications is utero-placental insufficiency. Aspirin has a wide range of pharmacological activities, including analgesic, antipyretic, antiplatelet and vasomotor properties.(9) The exact mechanism by which aspirin prevents preeclampsia is unknown, but it is thought that antithrombotic and vasodilator effects, and restoring the imbalance of thromboxane and prostacyclin play an important role by improving placentation.(9, 10) Anti-inflammatory effects have also been thought to contribute.(9, 10)

The optimal dose of aspirin has not been explored sufficiently. Current international guidelines advise aspirin doses of 75-150mg once daily to prevent preterm preeclampsia.(11-14) Recent meta-analyses suggest that aspirin doses above 100mg may be more effective than lower doses.(5, 15, 16) However, this hypothesis is based on indirect comparisons.

To evaluate the inhibitory effect of frequently used low-dose aspirin 80mg on platelet function, we performed an explorative study comparing aspirin 80mg once daily with placebo.

METHODS

Design and setting

In this explorative study, we performed platelet function tests in a subset of APRIL trial participants. The APRIL trial was a multicentre double-blind placebo-controlled randomized trial assessing the effect of aspirin 80mg once daily on the prevention of recurrent preterm birth.(17) The APRIL trial was funded by ZonMw, The Dutch Organization for Health Research and Development (grant number 836041006), and registered in the Dutch Clinical Trial Registry (NL5553, NTR5675). The study protocol and trial results have been described in detail.(17, 18)

The present sub-study was performed in the Amsterdam University Medical Centre, and was also approved by the Medical Research Ethics Committee (no. 2015_332). Women gave additional written informed consent for this study. No grant was received from funding agencies in the public, commercial, or not-for-profit sectors.

Participants

Our sub-study applied the same in- and exclusion criteria as the APRIL trial. The APRIL trial included women of 18 years and older with a singleton pregnancy and a history of spontaneous preterm birth between 22 and 37 weeks gestational age. Exclusion criteria were a previous indicated preterm birth for maternal or fetal reasons; other indications for aspirin use; thrombocytopenia or

thrombocytopathy; or major fetal malformations in the current pregnancy or in a prior pregnancy ending in spontaneous preterm birth.

Intervention

Women were randomized between aspirin 80mg once daily or placebo. Treatment was initiated between 8 and 16 weeks gestational age and continued until 36 weeks gestational age or delivery, if delivery occurred earlier. Women were instructed to take the medication in the evening.
Participants, their health care providers, and researchers were blinded for treatment allocation. The technician performing the platelet function tests was blinded for the use of aspirin or placebo, was unaware of the clinical condition and management of the patients, and was not involved in the analysis of the results of the APRIL trial. Allocation deblinding of the research team was performed after the completion of data collection.

Data collection

Venous blood samples were drawn at regular antenatal outpatient visits in the second trimester (between 18-22 weeks gestational age) and the third trimester (between 28-32 weeks gestational age) with an interval of at least eight weeks between measurements. Participants were instructed to take their study medication in the evening and to have a light breakfast on the day of blood sampling. Data on maternal characteristics, medical and obstetric history, platelet function tests, and pregnancy outcomes were recorded in an electronic case record form.
We evaluated overall medication adherence throughout pregnancy as well as medication adherence in the then days prior to blood sampling. Overall medication adherence was calculated by dividing the number of used tablets by the expected number of doses per participant. We considered overall medication adherence to be good if ≥80% of tablets were taken. At the time of blood sampling, the sub-study participants completed a structured interview including the number of tablets taken in the ten days prior to testing. If at least eight tablets were taken in the ten days prior to the test, women were considered adherent at the respective time points.

Laboratory techniques

Three different platelet function tests were used to analyze the blood samples since no gold standard for aspirin response in pregnancy exists: VerifyNow® point-of-care system (Accumetrics, CA, USA), Chronolog light transmittance aggregometry (LTA) and serum thromboxane B_2 (TxB_2) levels using an enzyme immunoassay kit (Assay Designs®, Ann Arbor, MI, USA). Blood samples for platelet function tests with VerifyNow® and Chronolog LTA were analyzed within three hours of collection. Maternal serum for TxB_2 measurements was prepared and stored within three hours of collection.

VerifyNow® measures the antiplatelet effect of aspirin along the inhibition of the cyclo-oxygenase-1 pathway by utilizing 1.0mM arachidonic acid.(19) Whole blood of the participant was tested. Change in light transmittance was measured and the results were displayed in Aspirin Reaction Units (ARU).

Chronolog LTA measures the light transmittance of platelet rich plasma stimulated by arachidonic acid.(20) Addition of arachidonic acid reduces formation of thromboxane A_2 (TxA_2) and thereby platelet aggregation. In the case of aspirin resistance, platelets aggregation is not reduced and the light transmittance is increased. Maximal percentage of aggregation was measured.

Serum TxB_2 is a direct measure of the capacity of platelets to synthesize TxA_2 and is a direct measure of the pharmacological effect of aspirin on platelets.(21) After the blood drawn, whole blood samples were stored in a stove at 37 degrees Celsius for one hour. Thereafter, samples were centrifuged with 3000 rotations per minute for ten minutes to create serum. Serum was stored at -80 degrees Celsius. At the end of the data collection, all serum samples were analyzed for TxB_2 by enzyme immunoassay in the laboratory for hematology, unit thrombosis and hemostasis of the Radboud University Medical Center in Nijmegen, the Netherlands.

Outcomes

The main outcomes of our sub-study were platelet function based on the three platelet function tests VerifyNow®, Chronolog LTA and serum TxB_2. Furthermore, secondary outcomes were preterm birth <37 weeks gestational age, pregnancy-induced hypertension, preeclampsia, birth weight, small-for-gestational age (<10th centile), and gestational age at birth.

Statistical analysis

This sub-study started while the RCT was well in progress. We could not perform an accurate sample size calculation due to large variations in measured platelet responses in earlier studies with the same test outcome measures.(22) The median and interquartile range (IQR) was calculated for all three platelet function tests for each time point, as well as the median and IQR of the average of the two time points. In case only one time point was available, the single measurement was used. The Wilcoxon signed rank test was performed to compare the measurements at the two time points and a p-value was reported. Differences in platelet function between treatment groups was calculated with the Mann Whitney U test by using the average of the two time points. We reported the differences in medians and the p-values. Statistical analyses were performed in SPSS version 26.0. P-values of less than 0.05 were considered to be statistically significant.

RESULTS

From September 2018 to May 2019, 11 participants of the APRIL study trial were included in the present study: six in the aspirin group and five in the placebo group. Baseline characteristics were similar between treatment groups (**Table 1**).

Two women in the aspirin group had one measurement instead of two measurements during pregnancy: one only in the second trimester and one only in the third trimester. The TxB_2 measurement in the second trimester of one participant in the aspirin group failed due to blood clotting. The results of the measurements per participant and the median of the measurements according to treatment group are illustrated in **Figure 1**. In the aspirin group, the measurements between the second and third trimester were comparable for all three platelet function tests (for all $p>0.05$, **Table 2**). In the placebo group, the serum TxB_2 levels were higher in the third trimester compared to the second trimester ($p=0.043$). The median differences of platelet function in the second and third trimester between allocation groups are shown in **Table 3**, all showing statistically significant lower levels in the aspirin group. As seen in **Figure 1**, there was one participant in the aspirin group without platelet inhibition in all three tests. She reported to be non-adherent to the study medication in both trimesters.

Overall medication adherence was good with a median of 99.4% in the aspirin group and 95.9% in the placebo group. Details on medication adherence prior to blood sampling are listed in **Table 4.** No adverse obstetric outcomes occurred in the aspirin group. In the placebo group, there were three preterm births and one small-for-gestational age neonate. None of the women developed preeclampsia.

DISCUSSION

Principal findings

This explorative study evaluated the effect of low-dose aspirin 80mg on platelet function with three platelet function tests in the second and third trimester of pregnancy in a placebo-controlled setting. Low-dose aspirin 80mg had a clear inhibitory effect on platelet function as assessed by VerifyNow®, Chronolog LTA and serum TxB_2 levels. Platelet function in the aspirin group was comparable in the second and third trimester of pregnancy.

Interpretation in light of other evidence

We demonstrated the clear inhibitory effect of aspirin 80mg on platelet function. These results are in line with a previous placebo-controlled study demonstrating a clear inhibitory effect of aspirin 60mg on TxB_2 levels in a population of 1002 pregnant women.(23) A longitudinal study evaluating the effect of aspirin 80mg on platelet function tests during the three trimesters of pregnancy and >3 months postpartum showed a more variable platelet response over time in individual women, but lacked a placebo group for comparison.(22)

In adult cardiology, the VerifyNow®, Chronolog LTA and serum TxB_2 assays have proven to be useful for the prediction of recurrent cardiovascular events in aspirin treated patients.(24) The present sample size was too small to determine whether the observed platelet response to aspirin 80mg relates to adverse obstetric outcome. Three previous studies did evaluate this relationship. Two studies did find an association between aspirin non-responsiveness and preeclampsia and fetal growth restriction, however, they did not take medication adherence into account.(21, 25) Navaratman et al, who did take medication adherence into account, found no association between aspirin non-responsiveness and adverse obstetric outcomes.(26)

The exact threshold for an adequate platelet response is currently unknown. Future studies should evaluate whether platelet function tests correlate with clinical outcomes in the obstetric population. Finding a gold standard with a clinical threshold would be useful to determine the optimal individual aspirin dose. In addition to an antiplatelet response, low-dose aspirin also has anti-inflammatory properties that are thought to contribute to improved pregnancy outcomes.(27) Low-dose aspirin of 81mg is known to increase the formation of anti-inflammatory mediator aspirin-triggered 15-epilipoxinA_4 (ATL).(28) In women at risk for developing preeclampsia, ATL plasma concentration is up to 70% lower than low-risk women.(27) Aspirin use during pregnancy resulted in higher ATL plasma concentration and lower incidence of preeclampsia, indicating an anti-inflammatory effect of aspirin on the development of preeclampsia.(27) Our study only addressed aspirin's effect on platelet function. Anti-inflammatory effects should also be considered when evaluating optimal aspirin dosing.

We found no evidence of laboratory aspirin resistance, but this could not be ruled out with the present sample size. In a recent study assessing aspirin 100-150mg in 220 high-risk pregnant women, none of the adherent women showed signs of laboratory aspirin resistance measured by PFA-100.(29) Also Mone et al. concluded that in case of confirmed biochemical adherence, aspirin non-responsiveness did not exist.(30) This raises the question whether laboratory aspirin resistance exists, or if a poor platelet response to low-dose aspirin is a result of medication non-adherence.

Medication adherence may play a more important role in the prophylactic treatment effect than variable platelet responses between individuals. Previous studies have shown that pregnant women with aspirin adherence ≥90% have a significantly improved treatment effect as compared to non-adherent women.(29, 31) Non-adherence to aspirin is reported to occur in up to 46% of pregnant women.(32) Efforts should be made to enhance medication adherence in pregnancy.

Obesity might also be a cause of poor platelet response. Finneran et al. have shown that high-risk obese women have less marked decrease of TxB_2 levels in response to aspirin 60mg as compared to non-obese women in pregnancy, suggesting that higher doses of aspirin may be necessary in the obese pregnant patient(23).

In our study, the levels of the VerifyNow® and Chronolog LTA were comparable between the second and third trimester. However, serum TxB_2 levels in the placebo group were higher in the third trimester as compared to the second trimester. The trend of rising TxB_2 levels during pregnancy has been reported before.(33) In the aspirin group, on the other hand, the TxB_2 levels were comparable during the second and third trimester of pregnancy. Aspirin may have diminished the rising TxB_2 levels. In the prevention of preeclampsia, it is thought that aspirin corrects the imbalance of prostacyclin and thromboxane.(34)

Strengths and limitations

This explorative study gives insight in the pharmacodynamic effects of an aspirin dose of 80mg in the second and third trimester of pregnancy using three platelet function tests. It was performed in an unique setting of a placebo-controlled double blind RCT. The platelet function tests were evaluated as continuous outcomes since there is no evidence supporting previously reported cut-off values for aspirin resistance in the pregnant population. We confirmed the great variety of outcomes between the different platelet function tests, similar to the observations in our prior study.(22) The striking difference between the aspirin and the placebo group overcomes the main limitation of the present study, namely the limited sample size. The latter, however, hinders the performance of a next step in the investigation: to relate platelet response to clinical obstetric outcome. Although this was known from the start of this sub-study.

Future

Despite the clear inhibitory effect of 80mg of aspirin on platelet function in this study, the pharmacodynamics and pharmacokinetics of aspirin in pregnancy, and the optimal aspirin dose to prevent obstetrics complications remain unknown. Future studies should further explore the pharmacology of different aspirin doses in pregnant women. Such studies should include platelet function tests, measurements of (anti-)inflammatory markers as for instance ATL, and detailed data on medication adherence, preferably determined by biochemical assays. It is also important to determine how factors such as body-mass index influence aspirin's pharmacokinetics. In addition, it may be evaluated whether a higher dosing frequency might result in improved platelet inhibition. In adult cardiology, there are indications that patients might benefit from a twice daily regimen of low-dose aspirin due to a more stable level of platelet inhibition.(35) However, increased dosing frequency may affect medication adherence.

CONCLUSION

Aspirin 80mg has a clear inhibitory effect on platelet function as assessed by VerifyNow®, Chronolog LTA and serum TxB_2. The on-aspirin platelet response appeared comparable in the second and third trimester of pregnancy.

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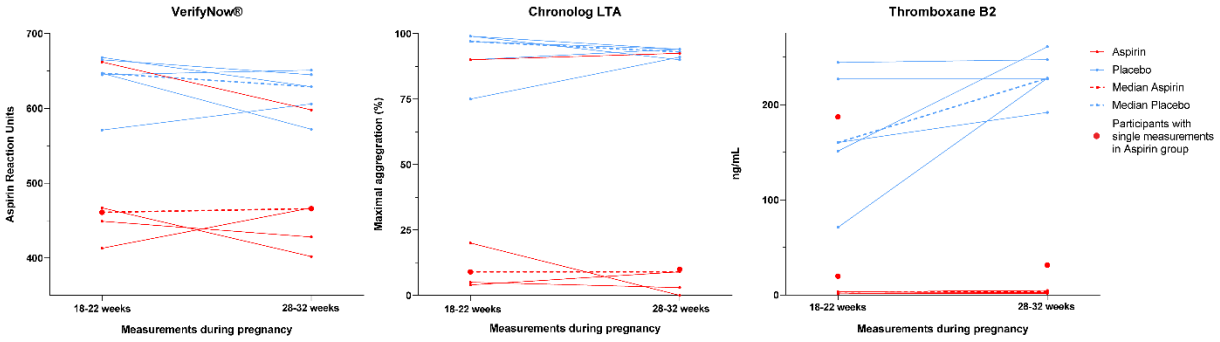

Figure 1 Platelet function tests during pregnancy

Table 1 *Baseline characteristics*

Characteristics	**Aspirin group (n = 6)**	**Placebo group (n = 5)**
Maternal age (years)	32.0 (28.3 – 33)	31.0 (27.5 – 34.5)
BMI (kg/m^2)	25.0 (20.4 – 25.3)	23.1 (20.8 – 26.4)
Ethnic origin		
White	4 (66.7%)	5 (100%)
Other	2 (33.3%)	0 (0%)
Smoking	0 (0%)	0 (0%)
Alcohol	0 (0%)	0 (0%)
Systolic blood pressure first trimester (mmHg)	103 (100 – 106)	105 (100 – 113)
Diastolic blood pressure first trimester (mmHg)	70 (64 – 70)	60 (54 – 63)
Obstetrics history		
Parity		
1	3 (50%)	5 (100%)
2	3 (50%)	0 (0%)
Gestational age of previous spontaneous preterm birth		
22^{+0} – 29^{+6} weeks	4 (66.7%)	3 (60.0%)
30^{+0} – 33^{+6} weeks	1 (16.7%)	1 (20.0%)
34^{+0} – 36^{+6} weeks	1 (16.7%)	1 (20.0%)

Data are presented as median (IQR) or numbers (%) as appropriate.

Table 2 *Comparison of platelet function in second and third trimester*

Platelet function test	Aspirin group (n = 6)			Placebo group (n = 5)		
	Second trimester (18-22 weeks)	Third trimester (28-32 weeks)	p-value paired samples	Second trimester (18-22 weeks)	Third trimester (28-32 weeks)	p-value paired samples
VerifyNow® (ARU)	461.0 (431.0-564.5)	466.0 (415.0-532.5)	0.273	647.0 (608.0-666.5)	629.0 (589.0-648.0)	0.345
Chronolog LTA (%)	9.0 (4.5-55.0)	9.0 (1.5-51.3)	1.000	97.0 (82.5-99.0)	92.5 (90.3-94.0)	0.893
Serum TxB_2 (ng/mL)	3.3 (2.1-103.4)	3.5 (2.0-24.7)	0.593	160.1 (111.1-235.6)	227.8 (177.6-254.1)	0.043

Data are depicted as median (IQR).

Table 3 *Comparison of platelet function between treatment groups*

Platelet function test	Median of second and third trimester			
	Aspirin (n = 6)	Placebo (n = 5)	Median difference	p-value
VerifyNow® (ARU)	450.5 (437.5-507.0)	648.0 (599.0-651.8)	197.5	0.017
Chronolog LTA (%)	9.5 (5.9-30.3)	94.5 (87.5-96.0)	85	0.009
Serum TxB_2 (ng/mL)	11.9 (2.4-70.3)	175.9 (161.6-236.6)	164	0.030

Data are depicted as median (IQR).

Table 4 *Medication use and adherence per treatment group*

	Aspirin group (n = 6)	Placebo group (n = 5)	p-value
Medication use			
Start medication (gestational age)	13^{+6} (11^{+5}-14^{+6})	14^{+1} (11^{+4}-15^{+4})	0.690
Stop medication (gestational age)	36^{+0} (34^{+6}-36^{+0})	35^{+0} (32^{+4}-36^{+0})	0.190

Overall medication adherence (%)	99.4% (94.2%-100%)	95.9% (90.7%-97.2%)	0.393
No. of adherent women 1st measurement	3/4 (75.0%)	5/5 (100%)	0.556
No. of adherent women 2nd measurement	3/5 (60.0%)	3/4 (75.0%)	0.730

Data are presented as median (IQR) of number (%) as appropriate.

HIGHLIGHTS

- Aspirin is used for prevention of preeclampsia and other obstetric complications.
- Aspirin treatment failure could be attributable to resistance or non-adherence.
- Three platelet function tests were performed in women assigned placebo or aspirin.
- Aspirin 80mg has a clear inhibitory effect on platelet function during pregnancy.
- The effect of aspirin on platelet function is similar in second and third trimester.