Pregnancy Hypertension 15 (2019) 141–145

Contents lists available at ScienceDirect

Pregnancy Hypertension

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

Aspirin causes endothelium-dependent vasodilation of resistance arteries from non-gravid and gravid rats

Helga Helgadottir[a,b], Teresa Tropea[b], Sveinbjorn Gizurarson[a], Hamutal Meiri[c], Maurizio Mandalà[b,*]

[a] Faculty of Pharmaceutical Sciences, University of Iceland, Hofsvallagata 53, 107 Reykjavik, Iceland
[b] Department of Biology, Ecology and Earth Sciences, University of Calabria, Arcavacata di Rende (CS), Italy
[c] TeleMarpe Ltd., Tel Aviv, Israel

A B S T R A C T

Objective: The objective of this study was to understand the effect of acetylsalicylic acid (aspirin) on resistance arteries from mesentery and uterus. During pregnancy, the uterine vasculature undergoes consistent growth to provide sufficient uteroplacental blood flow, a process whose failure is associated with pregnancy complications characterized by high uterine vascular resistance.

Methods: Uterine arcuate (UA) and mesenteric arteries (MA; diameter < 300 μm) isolated from non-gravid, mid-gravid (day 14), and late-gravid rats (day 20) were exposed to aspirin (10^{-12} to 10^{-5} M). Further, in UA from late-gravid rats, aspirin was evaluated in presence of inhibitors of nitric oxide synthases, cyclooxygenase, cyclic nucleotides (cAMP, cGMP) and BK channels, and also on endothelium-denuded vessels.

Results: Aspirin dilated both UA and MA in a dose dependent manner. Pregnancy increased aspirin vasodilation in MA and UA from mid-gravid rats, an effect that was reduced in vessels from late gravid animals at concentrations > 10^{-7} M. Further, uterine vasodilation was significantly reduced when the endothelium was removed ($p < 0.001$), and by inhibitors of nitric oxide synthase ($p < 0.001$), cyclooxygenase synthase ($p < 0.05$), cyclic nucleotides cGMP/cAMP and BK channels.

Conclusion: This is the first study to show a direct vasodilatory effect of aspirin on rat uterine artery that is mediated by a combination of cellular – primarily endothelial - mechanisms. Our results in UA suggest that the use of aspirin may be effective in enhancing uteroplacental blood flow, while its vasodilation effect on MA may lower peripheral resistance.

1. Introduction

Acetylsalicylic acid, best known by its first trade name aspirin, is one of the oldest and most frequently used drugs in the world. Aspirin has multiple physiological effects, one of which is the inhibition of eicosanoid biosynthesis [1], making it highly important in cardiovascular and anti-inflammatory treatments. Aspirin is a nonsteroidal anti-inflammatory drug (NSAID), but unlike other NSAIDs, it does not increase the risk of hypertension [2,3], on the contrary, it causes slight decrease in blood pressure [3–5]. By acetylating cyclooxygenases (COX) it prevents COX binding to arachidonic acid (AA), which inhibits the productions of prostanoids such as prostacyclins and thromboxane A2 [6]. It has also been shown that aspirin by acetylating endothelial nitric oxide (NO) synthase [7] enhancing the production of NO [8], therefore, may have a positive effect in preventing cardiovascular diseases.

Numerous clinical trials have been carried out, supporting the idea that low-dose aspirin may be helpful for pregnant women at high risk of developing preeclampsia (PE) and intrauterine growth restriction (IUGR) [9–12]. These pregnancy complications, PE and IUGR, affect pregnant women world-wide and are a major cause of maternal and perinatal mortality and morbidity. One of the complications that characterizes PE and IUGR is increased uterine vascular resistance associated with endothelial dysfunction.

Because reduced uteroplacental blood flow is a hallmark of both PE and IUGR, we hypothesized that aspirin may have direct vasodilatory actions on the uterine circulation, particularly on small resistance arteries. The results confirm this hypothesis and suggest that this drug could alleviate pregnancy complications associated with increases in uterine artery resistance, such as PE and IUGR.

2. Materials and methods

2.1. Materials

The physiological salt solution HEPES-PSS was freshly prepared for each experiment and comprised of: NaCl (141.8 mM), KCl (4.7 mM), $MgSO_4$ (1.7 mM), EDTA (0.5 mM), $CaCl_2$ (2.8 mM), HEPES (10.0 mM), KH_2PO_4 (1.2 mM), Glucose (5.0 mM). The pH was adjusted to 7.40 at

* Corresponding author.
E-mail address: m.mandala@unical.it (M. Mandalà).

https://doi.org/10.1016/j.preghy.2019.01.001
Received 27 February 2018; Received in revised form 7 December 2018; Accepted 9 January 2019
Available online 10 January 2019

37.0 °C with 10 M NaOH.

Chemicals: phenylephrine, Nω-nitro-L-arginine methyl ester (L-NAME), indometachin, paxillin, 1H-(1,2,4)oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) and, 9-(Tetrahydro-2-furanyl)-9H-purin-6-amine (SQ) were purchased from Sigma-Aldrich (Milan, Italy).

Acetylsalicylic acid (aspirin, Rhodine® 3118) was kindly provided by Novacyl, Lyon, France.

2.2. Animals & approvals

Sprague-Dawley female rats, 12–14 weeks old, were used in all experiments. Rats were housed at a temperature-controlled condition of 22 °C ± 2 °C and under a 12-hour light/dark cycle; food and water were provided *ad libitum*. Females were bred overnight in isolated pairs by the placement of a male rat. The first day of pregnancy was confirmed by the presence of a seminal plug on the following morning.

This study was approved by the local ethical committee at the University of Calabria and the Italian Ministry of Health. All experiments were conducted in accordance with the '3R principles' (www.nc3rs.org.uk) to reduce the number of animals and to optimize experimental protocols for obtaining maximum data from each tested animal, and with the European Guidelines for the care and use of laboratory animals (Directive 2010/63/EU).

2.3. Vasodilation evaluation

Uterine arcuate arteries (UA, diameter < 300 μm)) and third order mesenteric arteries (MA, diameter < 300 μm) were obtained from age-matched non-gravid (NP), mid-gravid (P14, 14 days after conception) and late-gravid (P20, 20 days after conception) rats. Following euthanasia by isoflurane and decapitation, the abdominal cavity was opened, and the uterus with its vasculature and the entire mesentery were excided and placed in a Petri dish containing cold (4 °C) physiological HEPES salt solution (HEPES-PSS). Arterial segments (1–2 mm long) were dissected free from connective and adipose tissue and transferred to the chamber of a small-vessel arteriograph. One end of the vessel was tied onto a glass cannula and flushed of any luminal contents by increasing pressure using a servo-null pressure system (Living Systems Instrumentation) prior to securing the distal end onto a second cannula.

All vessels were continuously superfused with HEPES-PSS at 37 °C and pH 7.4, pressurized to 50 mmHg and equilibrated for 45–60 min before beginning experimentation. Lumen diameter was measured by trans-illuminating each vessel segment, and using a video dimension analyzer (Living Systems Instrumentation) in conjunction with data-acquisition software (Ionoptix) to continuously record lumen diameter. Following equilibration, all vessels were pre-constricted with phenylephrine to produce a 40–50% [13] reduction in baseline diameter. Once constriction was achieved and the tissue was stable for about 10 min, aspirin was added in increasing concentration from 10^{-12} to 10^{-5} M while continually measuring changes in lumen diameter using a video dimension analyzer (Living Systems Instrumentation).

In some vessels, the endothelium was removed by air perfusion prior to equilibration, and the effectiveness of this procedure was confirmed by the absence of dilatation to acetylcholine (10^{-5} M).

2.4. Mechanism determination

The mechanism of action was investigated using the following pharmacological inhibitors: Nω-nitro-L-arginine methyl ester (L-NAME, 2×10^{-4} M) for nitric oxide synthase (NOS), Indomethacin (10^{-5} M) for COX, paxilline (10^{-5} M) for BK_{Ca} channel, 1H-(1,2,4)-oxadiazolo-[4,3-a]-quinoxalin-1-one (ODQ, 10^{-5} M) for guanylate cyclase and 9-(Tetrahydro-2-furanyl)-9H-purin-6-amine (SQ, 10^{-5} M) for adenylate cyclase. Vessels were pre-incubated with inhibitors for 20 min before pre-constriction with phenylephrine and the addition of aspirin.

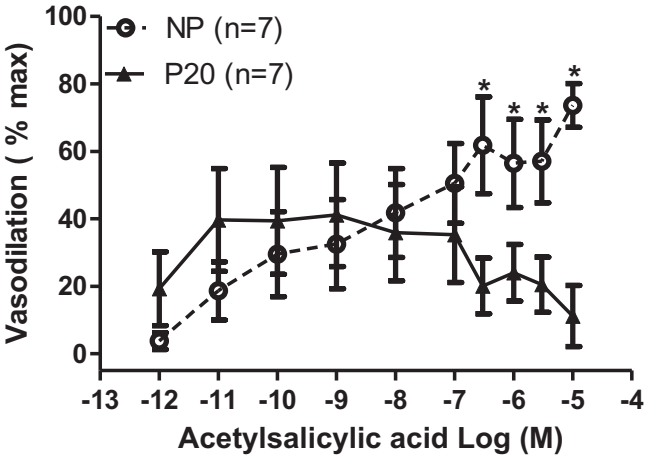

Fig. 1. Effect of Acetylsalicylic acid on uterine artery from non-gravid and late-gravid rats. Acetylsalicylic acid was tested on uterine arteries isolated from non-gravid (NP) and late-gravid rats (P20). Data are reported as Means ± SEM, n = number of experiments. Statistical analysis was performed using one-way ANOVA with Sidak's multiple-comparisons tests, $^{*}p < 0.05$.

2.5. Statistics and calculations

In pressurized arteries, vasodilation to aspirin was expressed as percent of maximal diameter which was determined at the end of each experiment by the addition of a relaxing PSS solution containing diltiazem (10 μM) and papaverine (100 μM).

Data are expressed as means ± SEM, where n is the number of arterial segments studied.

Data were analyzed for normal distribution by Shapiro-Wilk test. Differences in responses between groups were determined by one-way ANOVA with Sidak's multiple-comparisons tests or by Student's *t*-test, as indicated in figure legends. *p* values ≤0.05 were considered statistically significant.

3. Results

Aspirin induced vasodilation in UA isolated from both NP and P20 rats (Fig. 1). In NP rats the effect was concentration-dependent over the studied range (from 10^{-12} M to 10^{-5} M), with 75% of maximal vasodilation evident at the highest concentration. UA from P20 rats were more sensitive to aspirin at low concentrations, with a maximum vasodilatation of 40% up to 10^{-7} M; the effect decreased thereafter, and was only about 10% at 10^{-5} M, a significant reduction ($p < 0.05$) compared to the effect in NP rats.

UA from P14 rats showed a response to aspirin that was very similar to that in NP rats (Fig. 2) while, compared to vessels from P20 animals, the response was significant different ($p < 0.05$) at concentrations higher than 10^{-7} M.

To investigate whether the effect of aspirin was dependent on the type of vascular bed, aspirin was also tested on similarly-sized MA. The results in MA showed that aspirin induced a concentration-dependent vasodilation in both NP and P20 rats with a maximum vasodilatation of 20% and 60%, respectively ($p < 0.001$; Fig. 3).

To study the mechanism behind this effect in UA from P20 rats, a single intermediate concentration (10^{-10} M) that produced maximal vasodilation was used. As shown in Fig. 4, the absence of the endothelium significantly reduced the effect of aspirin ($p < 0.001$). When aspirin was tested in presence of L-NAME, a NO inhibitor, or indomethacin, a COX inhibitor, vasodilation was also reduced significantly ($p < 0.001$ and $p < 0.05$, respectively, as shown in Fig. 5). The results show that aspirin-induced vasodilation is highly dependent on NO.

As shown in Fig. 6, the effect of aspirin was also inhibited by the

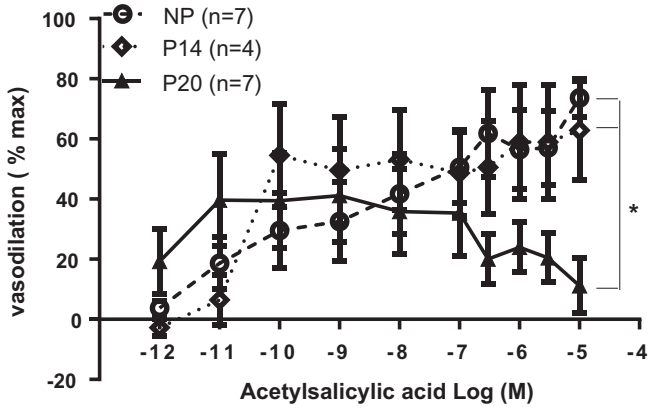

Fig. 2. Effect of Acetylsalicylic acid on uterine artery from non-gravid, mid-gravid and late-gravid rats. Acetylsalicylic acid was tested on uterine arteries isolated from non-gravid (NP), mid-gravid (P14) and late rats (P20). Data are reported as Means ± SEM, n = number of experiments. Statistical analysis was performed using one-way ANOVA with Sidak's multiple-comparisons tests, $^{*}p < 0.05$.

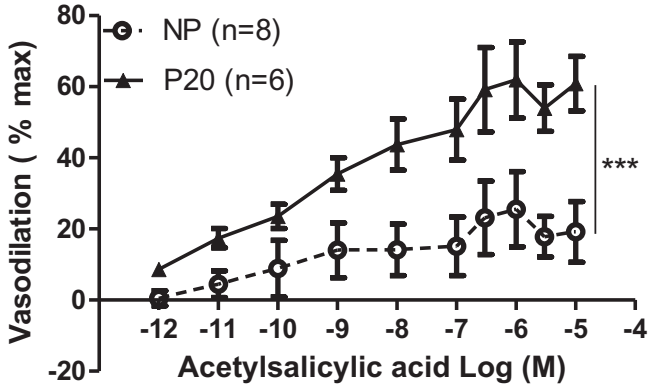

Fig. 3. Effect of Acetylsalicylic acid on mesenteric artery. Acetylsalicylic acid was tested on mesenteric arteries isolated from non-gravid (NP) and late-gravid rats (P20). Data are reported as Means ± SEM, n = number of experiments. Statistical analysis was performed using one-way ANOVA with Sidak's multiple-comparisons tests, $^{***}p < 0.001$.

cGMP inhibitor ODQ (50%, $p < 0.05$) and the cAMP inhibitor SQ (82%; $p < 0.01$) respectively. Furthermore, in presence of paxillin, a high-conductance Ca^{2+}-activated potassium channel (BK_{Ca}) inhibitor, vasodilation was reduced about 50% ($p < 0.05$, Fig. 7).

4. Discussion and conclusion

Our data show that aspirin induced a concentration-dependent vasodilation in UA, particularly in vessels from NP and P14 rats. While aspirin induced significant vasodilation at low concentrations (up to 10^{-7} M) in P20 rats, this effect declined significantly at higher concentrations. Interestingly, at the highest concentration of 10^{-5} M vasodilation was 6 times lower in P20 compared to NP and P14.

To find out if the effect of aspirin was specific to the uterine circulation, we exposed aspirin on MA from the splanchnic circulation, which accounts for about 40% of total peripheral resistance. Aspirin was also found to induce a concentration –dependent vasodilatation in MA and this effect was increased significantly in vessels from P20 rats, indicating that aspirin has a more pronounced effect in late-pregnancy, as compared to the non-pregnant condition.

In NP rats, aspirin induced a concentration-dependent vasodilation in both UA and MA, with a greater effect on UA (60–70%) than MA (20%). In P14 rats, the effect on UA was similar to that of NP rats. In

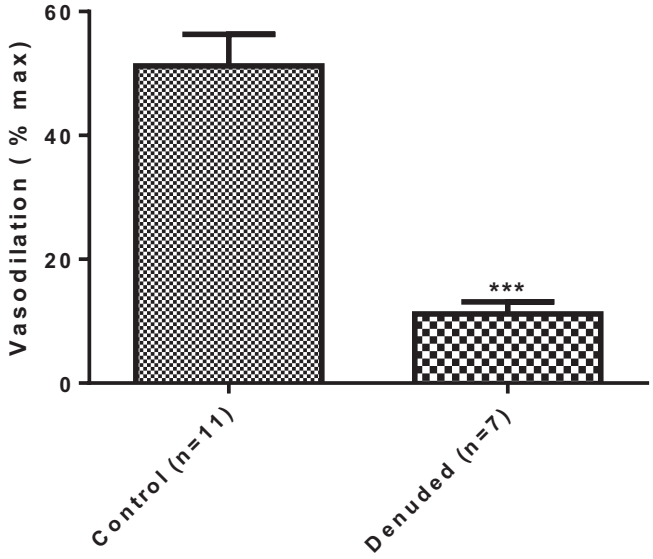

Fig. 4. Acetylsalicylic acid vasodilation is endothelium dependent. Acetylsalicylic acid at 10^{-10} M was tested on entire uterine arteries (control) and on uterine arteries without endothelium (denuded) isolated from late-gravid rats. Data are reported as Means ± SEM, n = number of experiments. Statistical analysis was performed using Student's *t*-test, $^{***}p < 0.001$.

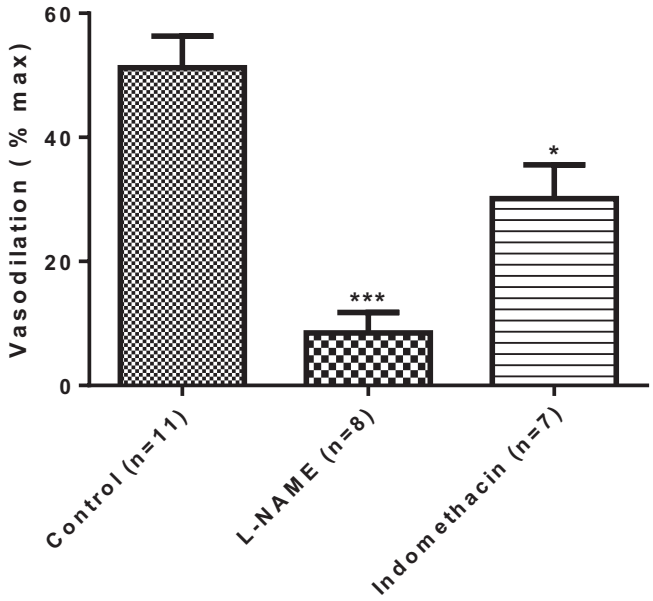

Fig. 5. Acetylsalicylic acid vasodilation is NOS and COX dependent. Acetylsalicylic acid at 10^{-10} M was tested on entire uterine arteries isolated from late-gravid rats in absence (control) and in presence of the NOS inhibitor (L-NAME) or the COX inhibitor (indomethacin). Data are reported as Mean ± SEM, n = number of experiments. Statistical analysis was performed using Student's *t*-test, $^{*}p < 0.05$, $^{***}p < 0.001$.

P20 rats, the effects of aspirin on UA faded at higher concentrations, contrary to MA, where it increased significantly. This difference in sensitivity of UA vs. MA to aspirin is highly dependent on whether the rats are pregnant or not. This differential response might be explained by the fact that the splanchnic and reproductive systems undergo different changes during pregnancy, e.g. the uterine vasculature, in particular, becomes less sensitive to vasodilators and undergoes considerable expansive remodeling [14]. Based on our results aspirin may, therefore, be beneficial to the cardiovascular system during pregnancy by increasing uteroplacental blood flow and lowering peripheral

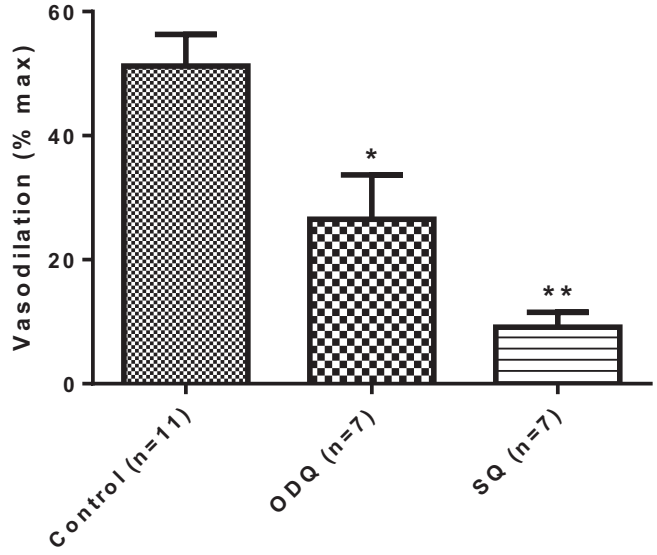

Fig. 6. Acetylsalicylic acid vasodilation is cyclic nucleotides dependent. Acetylsalicylic acid at 10^{-10} M was tested on entire uterine arteries, isolated from late-gravid rats, in absence (control) and in presence of the cGMP inhibitor (ODQ) or the cAMP inhibitor (SQ). Data are reported as Mean ± SEM, n = number of experiments. Statistical analysis was performed using Student's *t*-test, $^{*}p < 0.05$, $^{**}p < 0.01$.

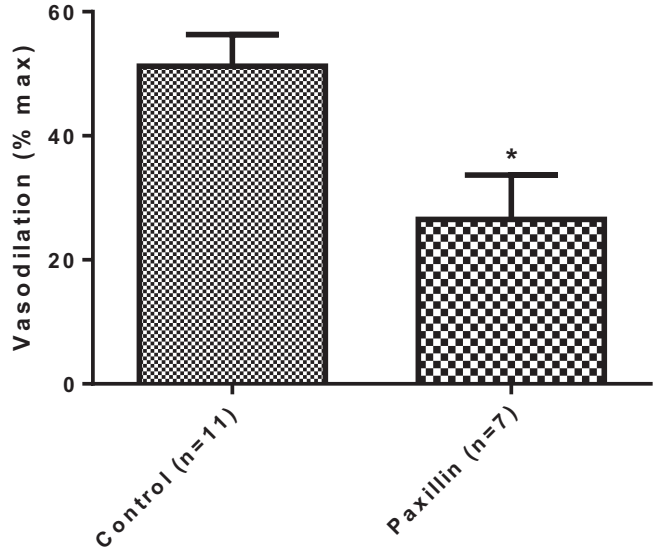

Fig. 7. Acetylsalicylic acid vasodilation is BK channels dependent. Acetylsalicylic acid at 10^{-10} M was tested on entire uterine arteries isolated from late-gravid rats in absence (control) and in presence of the BK channels inhibitor (paxillin). Data are reported as Mean ± SEM, n = number of experiments. Statistical analysis was performed using Student's *t*-test, $^{*}p < 0.05$.

resistance and blood pressure through its effects on resistance arteries from the uterine and splanchnic circulations, respectively.

Our results are particularly interesting in the light of a recent multicenter clinical study called ASPRE, showing that the administration of aspirin before week 16 of gestation to pregnant women at risk of developing PE prevents these women from developing preterm PE [15]. The maternal uterine circulation is a unique vascular bed that undergoes substantial vasodilation and growth during pregnancy [16] to provide adequate placental perfusion for normal fetal growth and pregnancy outcome. Failure of uterine vasculature adaptation to pregnancy increases the risk for maternal and fetal diseases such as preeclampsia and IUGR [17,18]. Our results show that aspirin-induced vasodilation of small UAs is dependent on both gestational age and concentration. This is in accordance with the ASPRE study [19] and a study from 2010, where Bujold et al. [9] evaluated clinical data on low- to mid- dose aspirin using meta-analysis, and showed that when the women started treatment on or before week 16, the risk of having PE or IUGR was significantly lowered compared to those who started aspirin administration after week 16. Furthermore, several clinical studies have shown that low-dose aspirin reduces uteroplacental vascular impedance in gestation [10,20].

Our results also correlate with the aspirin dosage recommendations, as the concentration of 10^{-7} 100–150 mg/day a dose suggested for treatment in women at risk for PE [15].

Although clinical studies have shown positive effect of aspirin on the uterine circulation, the exact pharmacological mechanisms remain elusive. Our results indicate that vasodilation induced by aspirin was reduced more than 80% when the endothelium was removed, suggesting that the effect is highly endothelial-dependent.

Inhibition of COX with indomethacin resulted in about a 40% reduction compared to control. This was surprising, since aspirin is known to inhibit COX, but perhaps the aspirin concentration tested was a too low dose for complete inhibition. In addition, a recent study showed a significant increase in UA COX in pregnant rats treated with low dose of aspirin [21], thus, this enzyme may be upregulated under these conditions, requiring higher aspirin levels for complete inhibition. Activation of COX likely contributes to aspirin-induced vasodilation through the production of prostacyclin.

The aspirin-induced vasodilation seems to be largely mediated by NO, as inhibition of NOS reduced the effects of aspirin by 80%, quantitatively similar to endothelial removal. NO is known to be a potent vasodilator of smooth vascular muscle via cGMP and other mechanisms, and plays a crucial role in pregnancy. In normal pregnancies the UA endothelium increases the production of NO compared to women who are not pregnant, while in women who develop PE, a reduction in NO signaling has been noted in a number of studies [22–24]. This raises the possibility that low-dose aspirin treatment can make up for the reduction in NO signaling that occurs in PE, and enhance vasodilation and uteroplacental blood flow.

In addition to COX and NOS inhibition, the inhibition of the cyclic nucleotides cGMP and cAMP reduced the vasodilatation induced by aspirin on UA in P20 rats by 50% and 75%, respectively. cAMP and cGMP act as second messengers and play an important role in regulating smooth muscle relaxation. It has been shown that in pregnancy the UA endothelium responds to cAMP by stimulating gap junction which enhances vasodilation [25] and regulating NO synthesis [26]. As already mentioned, it is also well known that vasodilation to NO involves increased smooth muscle cGMP an effect that underlies the utility of phosphodiesterase-5 inhibitors, which has also shown to be helpful in treating PE [27,28].

Finally, inhibition of BK channels was found to inhibit aspirin vasodilation by 50%. Since BK channels may be regulated by PKG, this observation further supports the primary importance of the NO/cGMP/BK mechanism in mediating the vasodilatory effects of aspirin on UA.

Aspirin has a very short half-life in the body (15–20 min) and is very rapidly hydrolyzed to salicylic acid (SA) [29]. We found that SA was also able to induce UA vasodilatation (data not shown), very similar to aspirin. That is interesting, since SA does not acetylate COX, and underlines the fact that aspirin-induced vasodilation is a complex mechanism mediated by several endothelial factors such as NO and prostacyclin acting upon vascular smooth muscle.

Other studies on different types of arteries have reported vasodilation induced by aspirin [30,31] but this is the first study to show a direct effect of aspirin on resistance UA, and to provide evidence for its mechanism being primarily endothelial-dependent and multifactorial in nature.

In summary, we show a powerful vasodilatory effect of aspirin on both UA and MA. Aspirin remains the single most effective and widely

used drug for the prevention of PE worldwide. The main manifestation of the disease is its negative effect on the overall vascular system, and our results support the rationale of using aspirin as a prophylactic treatment for women in risk for developing PE. Although it was beyond the scope of this study, it would be interesting to test low dose aspirin on UA isolated from pregnancies complicated by PE or IUGR to evaluate its vasodilatory effectiveness on vessels in the setting of gestational disease.

Acknowledgements

This study was supported by grants from the European Union 7th Framework Programme – FP7-HEALTH-2013-INNOVATION-2 (ASPRE Project # 601852), Hananja ehf, and by the Icelandic Research Fund.

A sincere thank you to Prof. George Osol for the proofreading of this paper, and to Dott. Alberto Montesanto for helping with the statistical analysis.

References

[1] S. Narumiya, Y. Sugimoto, F. Ushikubi, Prostanoid receptors: structures, properties, and functions, Physiol. Rev. 79 (4) (1999) 1193–1226.
[2] H. Vainio, G. Morgan, P. Elwood, The public health potential of aspirin, Pharmacol. Toxicol. 91 (2002) 49–50.
[3] A.T. Chan, J.E. Manson, C.M. Albert, C.U. Chae, K.M. Rexrode, G.C. Curhan, E.B. Rimm, W.C. Willett, C.S. Fuchs, Nonsteroidal antiinflammatory drugs, acetaminophen, and the risk of cardiovascular events, Circulation 113 (2006) 1578–1587.
[4] G.C. Curhan, A.J. Bullock, S.E. Hankinson, W.C. Willett, F.E. Speizer, M.J. Stampfer, Frequency of use of acetaminophen, nonsteroidal anti-inflammatory drugs, and aspirin in US women, Pharmacoepidem Drug Safety 11 (2002) 687–693.
[5] R.C. Hermida, D.E. Ayala, C. Calvo, J.E. López, Aspirin administered at bedtime, but not on awakening, has an effect on ambulatory blood pressure in hypertensive patients, J. Am. College Cardiol. 46 (2005) 975–983.
[6] G.J. Roth, D.C. Calverley, Aspirin, platelets, and thrombosis: theory and practice, Blood 83 (1994) 885–898.
[7] D. Taubert, et al., Aspirin induces nitric oxide release from vascular endothelium: a novel mechanism of action, Br. J. Pharmacol. 143 (1) (2004) 159–165.
[8] R.C. Hermida, et al., Differing administration time-dependent effects of aspirin on blood pressure in dipper and non-dipper hypertensives, Hypertension 46 (4) (2005) 1060–1068.
[9] E. Bujold, et al., Prevention of preeclampsia and intrauterine growth restriction with aspirin started in early pregnancy: a meta-analysis, Obstet Gynecol. 116 (2 Pt 1) (2010) 402–414.
[10] A.J. Crandon, D.M. Isherwood, Effect of aspirin on incidence of pre-eclampsia, Lancet 1 (8130) (1979) 1356.
[11] M. Haapsamo, H. Martikainen, J. Rasanen, Low-dose aspirin reduces uteroplacental vascular impedance in early and midgestation in IVF and ICSI patients: a randomized, placebo-controlled double-blind study, Ultrasound Obstet. Gynecol. 32 (5) (2008) 687–693.
[12] L.M. Askie, et al., Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data, Lancet 369 (9575) (2007) 1791–1798.
[13] I. Colton, M. Mandala, J. Morton, ST. Davidgeand, G. Osol, Influence of constriction, wall tension, smooth muscle activation and cellular deformation on rat resistance artery vasodilator reactivity, Cell Physiol. Biochem. 29 (5–6) (2012) 883–892.
[14] G. Osol, M. Mandala, Maternal uterine vascular remodeling during pregnancy, Physiology (Bethesda) 24 (2009) 58–71.
[15] D. Rolnik, et al., Aspirin versus placebo in pregnancies at high risk for preterm preeclampsia, N. England J. Med. 377 (7) (2017) 613–622.
[16] M. Mandala, G. Osol, Physiological remodelling of the maternal uterine circulation during pregnancy, Basic Clin. Pharmacol. Toxicol. 110 (1) (2012) 12–18.
[17] R. Becker, et al., Doppler sonography of uterine arteries at 20–23 weeks: risk assessment of adverse pregnancy outcome by quantification of impedance and notch, J. Perinat. Med. 30 (5) (2002) 388–394.
[18] L. Carbillon, First trimester uterine artery Doppler for the prediction of pre-eclampsia and foetal growth restriction, J. Matern. Fetal Neonatal Med. 25 (7) (2012) 877–883.
[19] N. Caron, et al., Low-dose ASA response using the PFA-100 in women with high-risk pregnancy, J. Obstet. Gynaecol. Can. 31 (11) (2009) 1022–1027.
[20] N. Lazzarin, et al., Low-dose aspirin and omega-3 fatty acids improve uterine artery blood flow velocity in women with recurrent miscarriage due to impaired uterine perfusion, Fertil. Steril. 92 (1) (2009) 296–300.
[21] O. Osikoya, et al., Effects of low-dose aspirin on maternal blood pressure and vascular function in an experimental model of gestational hypertension, Pharmacol. Res. 120 (2017) 267–278.
[22] C. Motta-Mejia, et al., Placental vesicles carry active endothelial nitric oxide synthase and their activity is reduced in preeclampsia, Hypertension 70 (2) (2017) 372–381.
[23] S.H. Nelson, et al., Increased nitric oxide synthase activity and expression in the human uterine artery during pregnancy, Circ. Res. 87 (5) (2000) 406–411.
[24] G. Osol, N.L. Ko, M. Mandalà, Altered endothelial nitric oxide signaling as a paradigm for maternal vascular maladaptation in preeclampsia, Curr. Hypertens. Rep. 19 (10) (2017) 82.
[25] B.C. Ampey, et al., Cyclic nucleotides differentially regulate Cx43 gap junction function in uterine artery endothelial cells from pregnant ewes, Hypertension 70 (2) (2017) 401–411.
[26] R.K. Dubey, D.G. Gillespie, E.K. Jackson, Adenosine inhibits collagen and protein synthesis in cardiac fibroblasts: role of A2B receptors, Hypertension 31 (4) (1998) 943–948.
[27] M. Wareing, et al., Phosphodiesterase-5 inhibitors and omental and placental small artery function in normal pregnancy and pre-eclampsia, Eur. J. Obstet. Gynecol. Reprod. Biol. 127 (1) (2006) 41–49.
[28] E.E. Gillis, J.N. Mooney, M.R. Garrett, J.P. Granger, J.M. Sasser, Sildenafil treatment ameliorates the maternal syndrome of preeclampsia and rescues fetal growth in the Dahl salt-sensitive rat, Hypertension 67 (3) (2016) 647–653.
[29] B.E. Cham, et al., Measurement and pharmacokinetics of acetylsalicylic acid by a novel high performance liquid chromatographic assay, Ther. Drug Monit. 2 (4) (1980) 365–372.
[30] Z. Ying, et al., Salicylates dilate blood vessels through inhibiting PYK2-mediated RhoA/Rho-kinase activation, Cardiovasc. Res. 83 (1) (2009) 155–162.
[31] P.Y. von der Weid, et al., Aspirin-triggered, cyclooxygenase-2-dependent lipoxin synthesis modulates vascular tone, Circulation 110 (10) (2004) 1320–1325.