**Effect of Low-dose and Standard-dose Aspirin on PGE2 Biosynthesis Among Individuals with Colorectal Adenomas: A Randomized Clinical Trial** – Supplemental Material

David A. Drew,1,2,3 Madeline M. Schuck,1,2 Marina Magicheva-Gupta,1,2 Kathleen O. Stewart,2 Katherine K. Gilpin,1,2 Patrick Miller,1,2 Melanie P. Parziale,1,2 Emily Pond,1,2 Oliver Takacsi-Nagy,1,2 Dylan C. Zerjav,1,2 Samantha M. Chin,1,2 Jennifer Mackinnon Krems,1,2 Dana Meixell,1,2 Amit D. Joshi,1,2 Wenjie Ma,1,2 Francis P. Colizzo III,2 Peter J. Carolan,2 Norman S. Nishioka,2 Kyle Staller,1,2 James M. Richter,2 Hamed Khalili,1,2 Manish Gala,1,2 John J. Garber,2 Daniel C. Chung,2 Joseph C. Yarze,2 Lawrence Zukerberg,4 Giovanna Petrucci,5 Bianca Rocca,5 Carlo Patrono,5 Ginger Milne,6 Molin Wang,3,7,8 Andrew T. Chan,1,2,8,†

**SUPPLEMENTAL METHODS**

**Urine sample processing and PGE-M and TXM measurements**

At each visit, at least 5 mL of urine was collected using sterile urine containers from all subjects. Urine samples were immediately placed on wet ice and transported to the laboratory. Within 1 hour of collection, urine samples were aliquoted on ice into 1.2 mL aliquots and stored at -80ºC until analysis. Urine samples were sent to processing laboratories by courier on dry ice and kept frozen until processing. The Eicosanoid Core Laboratory (PI: Ginger Milne) at Vanderbilt University measured PGE-M (11a-hydroxy-9, 15-dioxo-2,3,4,5-tetranor-prostane 1,20-dioic acid) levels in baseline/pre-treatment and post-treatment urine samples using liquid chromatography-mass spectrometry (LC/MS) as previously described.(1) For urinary PGE-M, 0.5mL of each urine specimen was stabilized by conversion to the *O-*methyloxime derivative and purified by C18 solid phase extraction with subsequent addition of the *O*-methyloxime derivatized deuterium-labeled internal standard (custom synthesis). Liquid chromatography (LC) was performed on an Acquity BEH C18 column (2.0 × 50 mm, 1.7μm particle, Waters Corporation, Milford, MA, USA) connected to a Waters Acquity I-Class UPLC system and delivered to a Waters Xevo TQ-S Micro triple quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA).

1 mL of urine sample was centrifuged at 340*g* for 10 minutes, the pellet was discarded and the supernatant was extracted by chromatography as previously described.(2) Urinary 11-dehydro-TXB2 was measured in the urine extracts by a standard Enzyme Linked Immunosorbent Assay (ELISA), as previously described, by using a specific rabbit polyclonal antibody(3). Results (ng/ml) were corrected for urinary creatinine, which was measured by a commercial kit (Creatinine Colorimetric Detection Kit, Enzo Life Sciences, Farmingdale, NY, USA) and final values were expressed as ng/mg creatinine.

**Serum sample processing and PGE2 and TXB2 measurements.**

In a subset of patients (n=30), 6 mL of blood was collected using a vacutainer that contained clot activator additive and no anticoagulant (Greiner Bio-One #456089) at both visits. The vacutainer was immediately placed in a 37ºC portable water bath and incubated for 1 hour. After 1 hour, vacutainers were centrifuged at 1200 x *g* for 15 minutes at room temperature and supernatant was transferred to 2 mL cryovials as 200 μL aliquots and stored at -80ºC until shipment. Serum PGE2 was measured by a standard ELISA using a specific commercial mouse monoclonal anti-PGE2 antibody (Cayman Chemicals, Ann Arbor, USA). Serum TXB2 was measured by a standard ELISA as previously described(4,5), using a specific rabbit anti-TXB2 polyclonal antibody(3).

**SUPPLEMENTAL REFERENCES**

1. Barnard ME, Beeghly-Fadiel A, Milne GL, Akam EY, Chan AT, Eliassen AH*, et al.* Urinary PGE-M Levels and Risk of Ovarian Cancer. Cancer Epi Biomarker Prev **2019**;28(11):1845 doi 10.1158/1055-9965.EPI-19-0597.

2. Pagliaccia F, Habib A, Pitocco D, Petrucci G, Zaccardi F, Di Stasio E*, et al.* Stability of urinary thromboxane A2 metabolites and adaptation of the extraction method to small urine volume. Clin Lab **2014**;60(1):105-11 doi 10.7754/clin.lab.2013.121238.

3. Pradelles P, Grassi J, Maclouf J. Enzyme immunoassays of eicosanoids using acetylcholine esterase as label: an alternative to radioimmunoassay. Analytical Chemistry **1985**;57(7):1170-3 doi 10.1021/ac00284a003.

4. Patrono C, Ciabattoni G, Pinca E, Pugliese F, Castrucci G, De Salvo A*, et al.* Low dose aspirin and inhibition of thromboxane B2 production in healthy subjects. Thrombosis Research **1980**;17(3):317-27 doi <https://doi.org/10.1016/0049-3848(80)90066-3>.

5. Rocca B, Santilli F, Pitocco D, Mucci L, Petrucci G, Vitacolonna E*, et al.* The recovery of platelet cyclooxygenase activity explains interindividual variability in responsiveness to low-dose aspirin in patients with and without diabetes. J Thromb Haemost **2012**;10(7):1220-30 doi 10.1111/j.1538-7836.2012.04723.x.

**A close up of a map

Description automatically generated**

**Supplemental Figure 1.** Thromboxane metabolite measurements in serum and urine of ASPIRED participants. A) Percent change in urinary TXM according to treatment assignment. B) Percent change in serum TXB2 according to treatment assignment. Mann-Whitney test, \*p<0.05; \*\*\*p <0.0001 C) Baseline measures of serum and urinary thromboxane metabolites are modestly correlated (Spearman r = 0.40; p=0.03) D) Change in urinary and serum thromboxane metabolites are modestly correlated (Spearman r=0.46; p=0.03).

**SUPPLEMENTAL TABLES**

|  |  |  |  |
| --- | --- | --- | --- |
| **Supplemental Table 1**. Minor adverse events and participant complaints according to treatment arm. | | | |
| **Minor Adverse Event / Participant Complaint** | **Placebo** | **Aspirin** | |
| **81 mg/day** | **325 mg/day** |
| GI upset (i.e. heartburn/acid reflux/nausea/gas) | 4 | 6 | 3 |
| Unrelated infection/cold symptoms/sinus related | 6 | 5 | 4 |
| Extended bleeding/bruising | 0 | 0 | 2 |
| Bleeding hemorrhoids | 0 | 0 | 1 |
| Constipation | 0 | 2 | 1 |
| Seasonal allergies | 1 | 2 | 3 |
| Fever | 1 | 0 | 2 |
| Headaches | 0 | 1 | 0 |
| Other, unrelated | 2 | 1 | 1 |
| Totals | 14 | 17 | 17 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Supplemental Table 2**. Median Urinary PGE-M measurements according to randomized intervention arm using non-parametric tests | | | | | |
|  |  | Aspirin dose assignment | | |  |
| Variable | **Placebo**  **(n=58)** | **81 mg/day**  **(n=57)** |  | **325 mg/day**  **(n=54)** | *PKruskal-wallis* |
| Baseline urinary PGE-M (ng/mg Cr) | 11.1 (8.0, 16.7) | 12.6 (8.4, 20.9) |  | 10.8 (8.0, 15.9) | 0.81 |
| Post-Intervention urinary PGE-M (ng/mg Cr) | 11.0 (7.0, 18.3) | 10.1 (4.9, 14.6) |  | 6.6 (4.2, 12.6)\*\* | 0.007 |
| ∆ urinary PGE-M (ng/mg Cr) | -0.3 (-2.8, 2.5) | -3.5 (-7.0, -0.2)\*\* |  | -3.4 (-5.7, -0.9)\*\* | 0.008 |
| % Change | -3.9 (-22.3, 32.1) | -26.8 (-48.7, -7.0)\*\* |  | -34.8 (-56.6, -10.3)\*\* | 0.00001 |
|  |  |  |  |  |  |
| Values are median (25th, 75th percentiles) unless otherwise noted.  Asterisks denote Mann-Whitney test comparing specific dose vs. placebo, \*\*p<0.001 | | | | | |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Supplemental Table 3**. Urinary PGE-M concentration according to randomized intervention arm after excluding two potential outliers | | | | | | |
|  |  | Aspirin dose assignment | | | |  |
| Variable | **Placebo**  (n=58) | **81 mg/day**  (n=56) | ***P81 v. placebo*** | **325 mg/day**  (n=53) | ***P325 v. placebo*** | ***Paspirin (grouped) v. placebo*** |
| Baseline urinary PGE-M, ng/mg Cr | 15.5 (12.6) | 16.0 (11.3) | *0.84* | 12.7 (7.3) | *0.15* | *0.51* |
| Post-Intervention urinary PGE-M, ng/mg Cr | 16.4 (15.8) | 13.2 (13.6) | *0.25* | 9.2 (7.7) | *0.003* | *0.016* |
| ∆ urinary PGE-M, ng/mg Cr | 0.8 (11.8) | -2.8 (11.8) | *0.10* | -3.6 (5.9) | *0.016* | ***0.018*** |
| % Change | 8.5 (50.6) | -14.1 (56.3) | *0.026* | -27.4 (40.1) | *<0.0001* | *0.0004* |
| The p-value for the primary outcome comparison is in **Bold**. Values are mean (SD) unless otherwise noted. P-values are generated from unpaired t-tests between groups, as noted by the subscript text, for each measure. No significant differences were observed between aspirin treatment groups (81 mg/day v. 325 mg/day), all p>0.05. | | | | | | |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Supplemental Table 4:** Association of selected variables with baseline urinary PGE-M and effect modification on effect of randomized treatment on change in PGE-M. | | | | |
|  | **Participants, n**  (N=180) | **Mean Baseline PGE-M**  (ng/mg Cr) | *Pbaseline*  (n=180) | *Pinteraction*  (n=169) |
|  |
| Age |  |  | 0.13c | 0.24c |
| <50 | 24 | 14.8 |  |  |
| 50-59 | 85 | 14.5 |  |  |
| 60-69 | 59 | 17.2 |  |  |
| 70+ | 12 | 20.7 |  |  |
| Sex |  |  | 0.07 | 0.28 |
| Male | 95 | 13.7 |  |  |
| Female | 85 | 17.8 |  |  |
| Race |  |  | 0.95 | 0.78 |
| White | 160 | 16.0 |  |  |
| Black/African American | 10 | 16.1 |  |  |
| Asian | 3 | 18.0 |  |  |
| More than one race | 6 | 11.2 |  |  |
| Did not report | 1 | 20.2 |  |  |
| Ethnicity |  |  | 0.53 | 0.96 |
| Not Hispanic/Latino | 175 | 15.8 |  |  |
| Hispanic/Latino | 5 | 19.9 |  |  |
| Marital Status |  |  | **0.02** | 0.83 |
| Married | 116 | 14.2 |  |  |
| Never married | 29 | 24.1 |  |  |
| Widowed | 10 | 20.2 |  |  |
| Separated | 3 | 14.2 |  |  |
| Divorced | 22 | 12.7 |  |  |
| Body Mass Index, kg/m2 |  |  | **0.04**c | 0.91c |
| Normal, <18.5-24.9 | 58 | 14.3 |  |  |
| Overweight, 25.0-29.9 | 75 | 11.0 |  |  |
| Obese, ≥30.0 | 47 | 21.4 |  |  |
| Smoking Status |  |  | 0.06 | 0.20 |
| Never | 106 | 14.4 |  |  |
| Former | 57 | 16.6 |  |  |
| Current | 15 | 24.2 |  |  |
| Alcohol Consumption |  |  | **0.04** | 0.91 |
| Never | 29 | 18.9 |  |  |
| Rarely | 48 | 12.4 |  |  |
| 1-5 times per week | 76 | 14.7 |  |  |
| Daily | 24 | 23.1 |  |  |
| More than daily | 3 | 21.9 |  |  |
| Personal Cancer History |  |  | 0.13 | 0.75 |
| No | 160 | 16.5 |  |  |
| Yes | 20 | 11.1 |  |  |
| Family History of CRC |  |  | 0.32 | 0.77 |
| No | 35 | 16.4 |  |  |
| Yes | 145 | 13.6 |  |  |
| Type II Diabetes |  |  | **0.0002** | 0.26 |
| No | 173 | 15.2 |  |  |
| Yes | 7 | 39.2 |  |  |
| Menopause Status (n=79) |  |  | 0.40 | 0.52 |
| Pre-menopausal | 17 | 9.6 |  |  |
| Peri-menopausal | 7 | 9.0 |  |  |
| Post-menopausal | 55 | 15.5 |  |  |
| History of 81 mg aspirin use |  |  | 0.08 | 0.94 |
| No, never | 158 | 15.3 |  |  |
| Yes, intermittently (<2x/wk) | 12 | 24.7 |  |  |
| Yes, regularly (>2x/week) | 6 | 9.4 |  |  |
| History of 325 mg aspirin use |  |  | 0.67 | 0.44 |
| No, never | 124 | 15.3 |  |  |
| Yes, intermittently (<2x/wk) | 49 | 16.8 |  |  |
| Yes, regularly (>2x/week) | 3 | 9.9 |  |  |
| History of NSAID use |  |  | **0.01** | 0.17 |
| No, never | 50 | 14.2 |  |  |
| Yes, intermittently (<2x/wk) | 29 | 17.7 |  |  |
| Yes, regularly (>2x/week) | 27 | 9.3 |  |  |
| PPI Use |  |  | 0.61 | 0.03 |
| Never | 132 | 16.6 |  |  |
| Intermittently | 13 | 15.7 |  |  |
| Currently and regularly | 19 | 12.8 |  |  |
| H2-Blocker Use |  |  | 0.23 | 0.49 |
| Never | 156 | 15.6 |  |  |
| Intermittently | 11 | 11.0 |  |  |
| **Supplemental Table 4 (continued)** | **Participants, n**  (N=180) | **Mean Baseline PGE-M**  (ng/mg Cr) | *Pbaseline*  (n=180) | *Pinteraction*  (n=169) |
| Currently and regularly | 9 | 29.6 |  |  |
| Statin Use, n |  |  | 0.31 | 0.86 |
| Never | 135 | 15.3 |  |  |
| Currently and regularly | 41 | 17.0 |  |  |
| Antacid Use, |  |  | 0.69 | 0.36 |
| Never | 145 | 15.8 |  |  |
| Intermittently | 23 | 14.9 |  |  |
| Currently and regularly | 11 | 19.6 |  |  |
| Indication for Previous Endoscopy |  |  | 0.83 | 0.38 |
| Screening | 98 | 15.8 |  |  |
| Surveillance | 44 | 14.1 |  |  |
| Diagnostic | 11 | 17.9 |  |  |
| Other/Unknown | 27 | 12.5 |  |  |
| Polyp History by location |  |  | 0.83 | 0.19 |
| Right | 74 | 16.9 |  |  |
| Left | 55 | 14.6 |  |  |
| Both | 50 | 15.9 |  |  |
| Unknown | 1 | 9.9 |  |  |
| Individuals with missing values were excluded from subsequent analysis of that variable.  a Association of variable with baseline urinary PGE-M level using general linear models (GLM). Model: PGE-Mbaseline = βvariable + β0  b P-value for multiplicative interaction term in GLM model: ΔPGE-M = βtreatment assignment + βvariable + β(variable\*treatment assignment) + βbaseline PGE-M + β0  c GLM uses continuous variable. | | | | |