The Effect of Dapagliflozin on Platelet Function Testing Profiles in Diabetic Patients: The EDGE Pilot Study

Naveen Seecheran · Kathryn Grimaldos · Kabeer Ali · Gabriella Grimaldos · Srivane Richard · Aleena Ishmael · Ceylon Gomes · Abhinav Karan · Rajeev Seecheran · Valmiki Seecheran · Sangeeta Persad · Harun Abdullah · Lakshmipathi Peram · Darren Dookeeram · Stanley Giddings · Shastri Motilal · Sadi Raza · Antonio Tello-Montoliu · David Schneider

ABSTRACT

Introduction: This prospective pharmacodynamic (PD) study assessed the effect of the sodium-glucose co-transporter-2 inhibitor (SGLT2i), dapagliflozin, on platelet reactivity.

Methods: Patients with stable coronary artery disease (CAD) and type 2 diabetes mellitus (T2DM) (n = 27) who were on maintenance dual antiplatelet therapy (DAPT) of aspirin 81 mg daily, and clopidogrel 75 mg daily were recruited. Platelet function was evaluated with the VerifyNow™ P2Y12 assay (Werfen, Bedford, MA, USA) and assessed prior to initiation of and after 10 days of treatment with dapagliflozin 10 mg once-daily dose regimen. Results were compared with a paired t test.

Results: Treatment with dapagliflozin significantly decreased P2Y12 reaction units (PRU) by 20%, (95% confidence interval (CI) 8.5–32.6%, p value 0.002). The mean difference in PRU was 36.70 (95% CI 16.66–56.75). No patients experienced any serious adverse events (SAEs).

Conclusions: Significantly diminished platelet reactivity was observed on dapagliflozin as compared to without dapagliflozin. This dedicated pharmacodynamic study could be potentially informative and applicable for Trinidadian stable CAD patients with T2DM on DAPT. Further studies are required to confirm these exploratory findings.

Clinical Trial Registration: EDGE ClinicalTrials.gov number NCT04400760.

Keywords: Dapagliflozin; Platelet reactivity; Platelet function; Sodium-glucose co-transporter 2 inhibitor (SGLT2i); VerifyNow™
Key Summary Points

Why carry out this study?
Sodium-glucose cotransporter-2 inhibitors (SGLT2i) have heralded a new paradigm in the management of diabetes mellitus (T2DM), heart failure (HF), and chronic kidney disease (CKD).

In a recent study, significantly attenuated platelet reactivity was observed on empagliflozin as compared to without empagliflozin.

Although this antiplatelet effect was demonstrated with empagliflozin, the question of whether this effect was either class-specific with respect to the general SGLT2i class or drug-specific to empagliflozin remained unanswered.

What was learned from the study?
Significantly diminished platelet reactivity was observed on dapagliflozin as compared to without dapagliflozin. Treatment with dapagliflozin significantly decreased P2Y12 Reaction Units (PRU) by 20%. The mean difference in PRU was 36.7.

This dedicated pharmacodynamic study could be potentially informative and applicable for Trinidadian stable CAD patients with T2DM on DAPT.

INTRODUCTION

Sodium-glucose cotransporter-2 inhibitors (SGLT2i) have heralded a new paradigm in the management of diabetes mellitus (T2DM), heart failure (HF), and chronic kidney disease (CKD) [1]. Recently, two seminal clinical trials; DAPA-HF (Study to Evaluate the Effect of Dapagliflozin on the Incidence of Worsening Heart Failure or Cardiovascular Death in Patients With Chronic Heart Failure) and EMPEROR-Reduced (EMPAgliflozin outcomeE tRial in Patients With chroNic heaRt Failure With Reduced Ejection Fraction) evaluating dapagliflozin and empagliflozin, respectively, reduced the risk of cardiovascular death and recurrent hospitalization for heart failure (HHF) by almost 25% in patients with heart failure with reduced ejection fraction (HFrEF) [2, 3].

SGLT2 inhibition attenuates hyperglycemia by inducing glucosuria [1]. Also, it promotes diuresis, with subsequent weight loss and anti-hypertensive effects [4]. There are also postulated anti-fibrotic effects and accentuated myocardial energetics efficiency [5]. In a recent study by our Thrombosis in Trinidad (TNT) group, The Effect of Empagliflozin on Platelet Function Profiles in Patients with Stable Coronary Artery Disease in Trinidad (EFFECT), significantly attenuated platelet reactivity was observed on empagliflozin as compared to without empagliflozin [6]. Although this antiplatelet effect was demonstrated with empagliflozin, the question of whether this effect was either class-specific with respect to the general SGLT2i class or drug-specific to empagliflozin remained unanswered. Therefore, we conducted this exploratory pilot study to assess the antiplatelet pharmacodynamic (PD) effect of dapagliflozin in a Trinidadian subpopulation with stable coronary artery disease (CAD) and T2DM.

MATERIALS

Study Design and Patient Population

The study complied with the Declaration of Helsinki, International Conference on Harmonization, Good Clinical Practice, and was approved by the Campus Research Ethics Committee of the University of the West Indies, St. Augustine, Trinidad [7]. All participants provided written informed consent to participate in a prospective, open-label study that assessed the effect of dapagliflozin 10 mg once daily for 10 days. Patients were screened and enrolled between May 2021 and July 2021 at the cardiology outpatient clinic at our institution, Trinidad Institute of Medical Technology.
They were considered eligible for the study if they were above 18 years of age and awaiting elective percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) on dual antiplatelet therapy (DAPT) for at least 4 weeks with aspirin 81 mg per day maintenance dose and clopidogrel 75 mg per day maintenance dose with T2DM. Exclusion criteria for this study included an acute coronary syndrome (ACS) within 6 months, active bleeding, prior hemorrhagic cerebrovascular event (CVE), clinical instability after an index event, use of an oral anticoagulation agent (warfarin derivative or other anticoagulant therapy such as dabigatran, rivaroxaban, apixaban, edoxaban), platelet count less than 100 x 10^9/μL, hemoglobin less than 10 g/dL, serum creatinine greater than 1.5 mg/dL, patients on concurrent CYP 2C19 inhibitors, and CYP 3A4 inducers. They were followed up for 28 days post-procedure after completing the study to assess whether they experienced any adverse events.

**Blood Sampling and VerifyNow™ P2Y_{12} Testing**

Clopidogrel was not administered on the morning of their fasting scheduled visit (8:00–9:00 am) (18–24 h before baseline blood sampling), which ensured the determination of clopidogrel-induced platelet reactivity (trough). Blood samples were obtained at rest by antecubital puncture using a 21-gauge needle and placed into Vacutette (Greiner Bio-One North America, Monroe, NC, USA) blood collecting tubes containing 3.8% trisodium citrate (#454322) after discarding the first 5 ml of blood to avoid artifactual platelet activation. Samples were processed by laboratory personnel blinded to ongoing study data. The platelet function assay utilized was the VerifyNow™ P2Y_{12} (VN-P2Y_{12}) assay (Werfen, Bedford, MA, USA). The assays were performed according to standard protocols, as previously described [6]. The VN-P2Y_{12} assay reports the results as P2Y_{12} reaction units (PRU). A PRU > 208 was considered high on-treatment platelet reactivity (HPR) according to the last consensus [8]. The enrolled patients were then treated with dapagliflozin 10 mg once daily for 10 days with pill accountability by the clinical research associate. After 10 days of the empagliflozin regimen, platelet reactivity was assessed a second time with the VN-P2Y_{12} assay using the aforementioned methodology (see Fig. 1).

**Patient Interview and Case Report Form**

The patients’ demographic data were recorded on a case report form (CRF) and included the patient’s medical, procedural history, and any cardiovascular medications.

**Statistical Analysis**

The sample size was calculated as 27 patients based on a paired proportion sample, an alpha (α) value of 0.05, power of 80%, estimated baseline prevalence of 40% of PRU > 208, and absolute delta of 20% (expected prevalence of 20% of PRU > 208). Continuous variables were expressed as means ± 95% confidence intervals and categorical variables as frequencies and

---

△ Adis
percentages. Paired $t$ tests were used to compare mean differences in PRU scores and McNemar’s test for paired proportions. No adjustments for multiple comparisons were made. Data collection was complete. A two-tailed $p$ value of 0.05 was considered to indicate a statistically significant difference for all the analyses performed. Statistical analysis was performed using SPSS version 25.0 software (IBM SPSS Statistics, New York, NY, USA).

**RESULTS**

A total of 27 patients with stable CAD and T2DM on DAPT with aspirin and clopidogrel were enrolled in the study. Table 1 shows the demographics of the study participants. The mean age was 63.5 years. Of the patients, 44% were females, and 74% were South Asian in ethnicity. The prevalence of prior myocardial infarction (MI) was 11%. The mean body mass index (BMI) was 26.9 kg/m$^2$. Thirty-seven percent were on insulin therapy, while 69% and 50% were on metformin and sulfonylureas, respectively. Fifty-two percent received percutaneous coronary intervention (PCI), with 26% undergoing coronary artery bypass grafting (CABG). The mean PRU on dapagliflozin was significantly less than without dapagliflozin at baseline (160.93, 95% confidence interval (CI) 129.28–192.57; vs. 197.63, CI 170.82–224.44, $p$ value 0.001) (Fig. 2). The mean difference in PRU was 36.70 (95% CI 16.66–56.75) which significantly resulted in inhibition of platelet aggregation (IPA) by 20%, (95% CI 8.5–32.6%, $p$ value 0.002). Forty-eight percent of patients had high on-treatment platelet reactivity with a PRU > 208, which decreased to 25.9% ($p$ value 0.07) with near-significance (Table 2). No patients experienced any serious adverse events.

**DISCUSSION**

The advent of SGLT2i has heralded a new era of the “triple threat” paradigm in managing T2DM, HF, and CKD. They have been incorporated into the HF armamentarium as a foundational pillar of the “fantastic four” [9].

| Table 1 Patient population | Characteristics | Frequency (%) |
|-----------------------------|-----------------|--------------|
|                             | Age             | 63.5 years (mean) |
|                             | Gender          |              |
|                             | Female          | 12 (44)      |
|                             | Male            | 15 (56)      |
|                             | Ethnicity       |              |
|                             | South Asian     | 20 (74)      |
|                             | Caribbean Black | 4 (15)       |
|                             | Interracial     | 3 (11)       |
|                             | Body mass index (BMI) | 26.9 kg/m$^2$ (mean) (normal 18.5–24.9 kg/m$^2$) |
|                             | Weight          | 73.1 kg      |
|                             | Systolic blood pressure | 149 mmHg (normal < 120 mmHg) |
|                             | Diastolic blood pressure | 83 mmHg (normal < 80 mmHg) |
|                             | Comorbidities   |              |
|                             | Prior myocardial infarction (MI) | 3 (11) |
|                             | Diabetes mellitus (DM) | 27 (100) |
|                             | Glycosylated hemoglobin (HbA$_1c$) | 9.13% (mean) (normal < 6%) |
|                             | Fasting blood glucose (FBG) | 194 mg/dl (normal < 126 mg/dl) |
|                             | Hypertension (HTN) | 25 (93) |
|                             | Dyslipidemia    | 14 (52)      |
|                             | Chronic kidney disease (CKD) | 0 (0) |
|                             | Cerebrovascular events (CVE) | 13 (48) |
|                             | Chronic obstructive pulmonary disease (COPD) | 2 (7) |
|                             | Peripheral artery disease (PAD) | 3 (11) |
|                             | Cardiovascular medications |              |
Compared to empagliflozin, which was evaluated in the EFFECT study, dapagliflozin is associated with more than 1200-fold higher potency for SGLT2 than SGLT1, lower plasma aldosterone, and norepinephrine levels [10]. A Japanese study also reported that dapagliflozin suppressed atherogenic small dense low-density lipoprotein while increasing high-density lipoprotein, a favorable cardiometabolic marker [11]. Dapagliflozin is also associated with lower fasting blood glucose levels and body weight, albeit at the expense of increased urinary tract infections [12].

Diabetes is intricately linked to a prothrombotic milieu that augments platelet reactivity [13]. Several key contributory factors include hyperglycemia, dyslipidemia, insulin resistance with resultant oxidative stress, inflammation, and endothelial dysfunction [14]. As outlined in the EFFECT trial, it was postulated that SGLT2i-mediated attenuation in platelet reactivity occurred via the multifaceted pathways of decreased hyperglycemia, dyslipidemia, obesity, insulin resistance, oxidative stress, inflammation, and endothelial dysfunction [6]. In the EFFECT study, the mean P2Y12 reaction units

| Characteristics                        | Frequency (%) |
|----------------------------------------|---------------|
| Aspirin                                | 27 (100)      |
| Clopidogrel                            | 27 (100)      |
| Angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, neprilysin inhibitor (ACEi, ARB, Ni) | 25 (93) |
| Beta-blocker (BB)                      | 18 (67)       |
| Statin                                 | 25 (93)       |
| Mineralocorticoid receptor antagonist (MRA) | 2 (7) |
| Calcium channel blocker (CCB)          | 7 (26)        |
| Nitrates                               | 14 (52)       |
| Ivabradine                             | 5 (19)        |
| Trimetazidine                          | 14 (52)       |
| Diabetic medications                   |               |
| Insulins                               | 10 (37)       |
| Oral hypoglycemics                     |               |
| Metformin                              | 18 (69)       |
| Sulfonylureas                          | 10 (50)       |
| Glucagon-like peptide-1 receptor agonists (GLP-1RA) | 7 (26) |
| Dipeptidyl peptidase-4 inhibitors (DPP-4i) | 0 (0) |
| Cardiovascular procedures              |               |
| Percutaneous coronary intervention (PCI) | 14 (52) |
| Coronary artery bypass grafting (CABG) | 7 (26) |
| P2Y12 reaction units (PRU)             |               |
| PRU > 208                              | 13 (48)       |
| PRU < 208                              | 14 (52)       |

| Characteristics                        | Frequency (%) |
|----------------------------------------|---------------|
| Serum hemoglobin (Hb)                  | 13.9 (normal 13.2–17.6 g/dl) |
| Serum creatinine (sCr) mg/dl           | 0.84 (normal 0.81–1.21 mg/dl) |
| Serum triglycerides (TG) mg/dl         | 197 (normal < 150 mg/dl) |
| Serum total cholesterol (TC) mg/dl     | 204 (normal < 170 mg/dl) |
| Serum low-density-lipoprotein (LDL) mg/dl | 169 (normal < 130 mg/dl) |
| Serum high-density-lipoprotein (HDL) mg/dl | 35 (normal > 50 mg/dl) |

Table 1 continued
(PRU) on empagliflozin were significantly less than without empagliflozin at baseline (187.35, 95% CI 155.38–219.32 vs. 217.25, CI 180.60–253.90; \( p \)-value < 0.030). The mean difference in PRU was 29.90 (95% CI 3.17–56.63). Seventy-five percent of patients had HPR with a PRU \( \leq 208 \), which decreased to half with near-significance (\( p \)-value 0.06) [6]. The mean PRU on dapagliflozin was significantly less than without dapagliflozin at baseline (160.93, 95% CI 129.28–192.57; vs. 197.63, CI 170.82–224.44, \( p \)-value 0.001). The mean difference in PRU was 36.70 (95% CI 16.66–56.75). Almost half of the patients had high HPR with a PRU > 208, which decreased slightly above one-quarter with near-significance (\( p \)-value 0.07). Both the mean differences in PRU were similar, in addition to the proportion of patients with HPR, which subsequently improved. This alludes to a possible signal that there is SGLT2i-class-effect on platelet reactivity as opposed to a drug-specific effect. While this study supports the EFFECT study in lending credence to either direct or pleiotropic attenuation in platelet reactivity, it does not fully elucidate the mechanistic effects of such.

### Study Limitations

Although this study was adequately powered for PD PRU outcomes, it was not designed for clinical endpoints, and as such, no clinical effectiveness or safety conclusions can be

### Table 2

Comparison of patients’ P2Y\(_{12}\) reaction units (PRU) and the percentage of high on-treatment platelet reactivity before and after dapagliflozin 10 mg

|                  | Mean platelet reaction units (PRU) | Lower 95% confidence interval (CI) | Upper 95% confidence interval (CI) | \( p \)-value | High on-treatment platelet reactivity (HPR) % | \( p \)-value |
|------------------|-----------------------------------|-----------------------------------|-----------------------------------|--------------|---------------------------------|--------------|
| Baseline         | 197.63                            | 170.82                            | 224.44                            | 0.001        | 48.1                            | 0.07         |
| Dapagliflozin    | 160.93                            | 129.28                            | 192.57                            | 25.9         |                                 |              |

*Fig. 2* Comparison of patients’ P2Y\(_{12}\) reaction units (PRU) before and after dapagliflozin 10 mg
drawn. As with previous studies conducted by this group in Trinidad, again, there was a preponderance of South Asian patients, alluding to a selection bias during study enrolment [6]. This study is predicated on the effect of dapagliflozin in stable CAD patients on DAPT with clopidogrel and may not be clinically pertinent to patients on more potent antithrombotic therapies such as ticagrelor or direct oral anticoagulants (DOACs). Another limitation is that only one modality of platelet function testing, the VN-P2Y12, was utilized with its inherent issues of variability in platelet aggregation over time and intra-assay variability. A more detailed, comprehensive panel of platelet function testing, including flow cytometry to assess Fcγ receptor type IIa (FcγRIIa), may prove to be complementary; however, these are not currently available in Trinidad for technical and personnel logistical reasons.

CONCLUSIONS

Dapagliflozin achieved a greater antiplatelet effect and significantly lower platelet reactivity than in Trinidadian patients with CAD and T2DM without dapagliflozin. This dedicated pharmacodynamic study could be potentially informative and applicable for Trinidadian stable CAD patients with T2DM on DAPT.

ACKNOWLEDGEMENTS

Disclosures. Naveen Seecheran, Kathryn Grimaldos, Kabeer Ali, Gabriella Grimaldos, Srivane Richard, Aleena Ishmael, Ceylon Gomes, Abhinav Karan, Rajeev Seecheran, Valmiki Seecheran, Sangeeta Persad, Harun Abdullah, Lakshmiapathi Peram, Darren Dookeeram, Stanley Giddings, Shastri Motilal, Sadi Raza, Antonio Tello-Montoliu, and David Schneider contributed equally to writing the manuscript, and read and approved the final manuscript.

Funding. The Rapid Service Fee was funded by the authors.

Compliance with Ethics Guidelines. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the Campus Research Ethics Committee of the University of the West Indies, St. Augustine, Trinidad (CREC-SA.0284/03/2020). All participants provided written informed consent to participate.

Data Availability. All available data can be obtained by contacting the corresponding author. EDGE ClinicalTrials.gov number NCT04400760. All materials, data, code, and associated protocols will be made promptly available to the editor and readers upon request. If requested, there will not be any restrictions on the availability of materials.

Open Access. This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License, which permits any non-commercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory
regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc/4.0/.

REFERENCES

1. Das SR, Everett BM, Birtcher KK, Brown JM, Januzzi JL, Kalyani RR, et al. Expert consensus decision pathway on novel therapies for cardiovascular risk reduction in patients with type 2 diabetes. J Am Coll Cardiol. 2020. https://doi.org/10.1016/j.jacc.2020.05.037.

2. McMurray JJV, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, et al. Dapagliflozin in patients with heart failure and reduced ejection fraction. N Engl J Med. 2019;381:1995–2008.

3. Packer M, Anker SD, Butler J, Filippatos G, Pocock S, Carson P, et al. Cardiovascular and renal outcomes with empagliflozin in heart failure. N Engl J Med. 2020;383:1413–24.

4. Vallon V, Thomson SC. Targeting renal glucose reabsorption to treat hyperglycaemia: the pleiotropic effects of SGLT2 inhibition. Diabetologia. 2017. https://doi.org/10.1007/s00125-016-4157-3.

5. Verma S, McMurray JJV. SGLT2 inhibitors and mechanisms of cardiovascular benefit: a state-of-the-art review. Diabetologia. 2018. https://doi.org/10.1007/s00125-018-4670-7.

6. Seecheran N, Ramdeen A, Debideen N, Ali K, Grimaldos K, Grimaldos G, et al. The effect of empagliflozin on platelet function profiles in patients with stable coronary artery disease in Trinidad: the EFFECT pilot study. Cardiol Ther. 2021;10:189–99.

7. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310:2191–4.

8. Tantry US, Bonello L, Aradi D, Price MJ, Jeong Y-H, Angiolillo DJ, et al. Consensus and update on the definition of on-treatment platelet reactivity to adenosine diphosphate associated with ischemia and bleeding. J Am Coll Cardiol. 2013. https://doi.org/10.1016/j.jacc.2013.07.101.

9. Bauersachs J. Heart failure drug treatment: the fantastic four. Eur Heart J. 2021;42(6):681–3. https://doi.org/10.1093/eurheartj/ehaa1012.

10. Nakagaito M, Joho S, Ushijima R, Nakamura M, Kinugawa K. Comparison of canagliflozin, dapagliflozin and empagliflozin added to heart failure treatment in decompensated heart failure patients with type 2 diabetes mellitus. Circ Rep. 2019;1:405–13.

11. Hayashi T, Fukui T, Nakanishi N, Yamamoto S, Tomoyasu M, Osamura A, et al. Dapagliflozin decreases small dense low-density lipoprotein-c-cholesterol and increases high-density lipoprotein-2-cholesterol in patients with type 2 diabetes: comparison with sitagliptin. Cardiovasc Diabetol. 2017;16:8.

12. Donnan JR, Grandy CA, Chibrikov E, PharmD CM, Aubrey-Bassler K, Johnston K, et al. Dose response of sodium glucose cotransporter-2 inhibitors in relation to urinary tract infections: a systematic review and network meta-analysis of randomized controlled trials. CMJ Open. 2018;6:E594–602.

13. Schneider DJ. Factors contributing to increased platelet reactivity in people with diabetes. Diabetes Care. 2009;32:525–7.

14. Keating FK, Sobel BE, Schneider DJ. Effects of increased concentrations of glucose on platelet reactivity in healthy subjects and in patients with and without diabetes mellitus. Am J Cardiol. 2003;92:1362–5.