Anthocyanins: Possible Antiplatelet Alternative for Aspirin Resistant Diabetic Population
Dr Indu Singh, Prof Peter Davoren, Prof Alfred Lam, Dr Natalie Colson

Proposal Background
The world prevalence of diabetes (T2DM), the fifth leading cause of death, among adults (aged 20–79 years) is predicted to rise to 7.7%, affecting 439 million adults by 2030 from approximately 6.4%, affecting 285 million in 2010 [1,2]. Cardio vascular disease (CVD), resulting from accelerated atherosclerosis, is responsible for about 70% of all causes of death in diabetics [3]. Endothelial dysfunction (ED) linked to obesity, T2DM and insulin resistance plays an important role in the pathogenesis and clinical expression of atherosclerosis [4]. Endothelial production of pro-thrombotic molecules (plasminogen activator inhibitor-1, thromboxane, tissue factor and von Willibrand’s factor) is balanced by the production of antithrombotic molecules such as nitric oxide (NO), heparin, prostacyclin, tissue plasminogen activator and thrombomodulin. This balance is shifted towards a prothrombotic and antifibrinolytic state in diabetic and pre-diabetic populations. The increased oxidative stress (OS) impairs NO bioavailability leading to ED.

Increased production of thromboxane A2 (TXA2) from arachidonic acid metabolism in diabetics increases platelet activation, aggregation and adhesion [5]. Platelets and leukocytes of T2DM patients are hyper reactive and express more adhesion molecules for circulating leukocytes [6]. Platelet P-selectin interacts with leukocyte P-selectin glycoprotein ligand (PSGL-1) leading to the formation of platelet leukocyte aggregates (PLA) [7 & 8]. Activated leukocytes secrete several pro-inflammatory cytokines and express a prothrombotic membrane phenotype.

Three classes of antiplatelet agents include cyclooxygenase-1 (COX-1) inhibitors (aspirin), ADP P2Y12 receptor antagonists (thienopyridines), and platelet GP IIb/IIIa inhibitors [9]. Aspirin (ASA) selectively acetylates the COX-1 enzyme, thereby blocking TXA2 synthesis in platelets [15]. Aspirin use for prevention of ischemic events in patients with or at risk of diabetes has been controversial [10–12]. Insulin resistant prediabetics respond poorly to ASA leading to atherothrombosis [13].

Antiplatelet drug resistance has emerged as a new concept and is responsible for some of the treatment failures [14]. Aspirin resistance is the lack of inhibition of the COX-1-mediated TXA2 pathway. Persistent high platelet reactivity with antiplatelet therapy is more frequent in diabetic compared with non-diabetic individuals [15, 16]. Diabetics taking low dose aspirin experience a 55% increased risk compared with those without diabetes [13]. The lack of beneficial effect of aspirin may be partly explained by its significant association with high HbA1c levels ≥8% [17].

Platelet hyper activity and recurrent thrombotic events in T2DM patients warrant for more potent antithrombotic therapies. We found QGP attenuates platelet activity targeting the COX-1 pathway under exercise induced OS in healthy population. This project will translate and evaluate the effect of anthocyanin in the diabetic population.

RESEARCH PLAN, APPROACH & SIGNIFICANCE:
Our hypothesis is that increased OS in diabetes is responsible for aspirin resistance. Antioxidants in QGP acting on same target as aspirin in platelet activation (COX-1 pathway) have the potential to replace or complement low dose aspirin. This project aims to compare the effect of QGP with low dose aspirin to evaluate the feasibility of using these as antiplatelet therapy in population resistant or non-responsive to aspirin.
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Randomized, double blind, cross over intervention trial

**Methods:**

- Normal healthy - Age & gender matched
- Diabetics - without prior clinical CVD
- Diabetics with prior clinical CVD
- Diabetics - at risk population – sedentary & obese

<table>
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<tr>
<th>Pre &amp; Post Supplementation Tests</th>
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<td>[Total 400 samples per test]</td>
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A final sample size of 25 volunteers in each group is required for 80% power to detect a 5% variation in the platelet activation, where a 3-5% standard deviation exists in the population, assuming an alpha error of 0.05.

**Significance**

CVD is the leading cause of morbidity and mortality in T2DM. Aspirin blocks platelet TXA2 synthesis and reduces platelet activity. Various antioxidants including QGP target same pathway as aspirin in attenuating platelet activity. However obese and diabetic populations are often non-responsive to ASA treatment leading to increased rate of mortality due to CVD.

This project will compare the effect of the QGP with aspirin in diabetics, prediabetics and normal healthy population by analysing platelet, PLA and aspirin resistance parameters. The results obtained will assist in future mechanistic studies to develop anthocyanin as antiplatelet therapy to complement or replace aspirin. The outcome of this project will significantly support therapeutic alternative to ASA, leading to effective prevention and treatment of oxidative stress induced metabolic syndrome conditions, providing fiscal and health benefit to society.

**REFERENCES:**

- Randomized, double blind, cross over intervention trial
- Diabetics
  - without prior clinical CVD
  - at risk population – sedentary & obese
  - N=100 (25 each group) (age 25-75)
- Normal healthy
  - Age & gender matched
- Blood tests:
  1. Full blood examination
  2. Insulin, glucose, HbA1c (HOMA-IR) [18]
  3. Platelet aggregation
  4. Flowcytometry for Activated platelet surface markers – CD62p/Pselectin, PAC-1
  5. Platelet – leucocyte aggregates by Flowcytometry
  6. Inflammation markers, CRP-HS, B-thromboxane
  7. Biochemical parameters – Lipid profile, uric acid, creatinine

**Urine samples:**

1. TXAB2, microalbumin & creatinine (to evaluate aspirin resistance) [19]
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