Pharmacological characterization of the sodium-glucose co-transporter 2 inhibitors, gliflozins in isolated platelets from healthy volunteers

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Abstract
Gliflozins are a new pharmacological class of drug approved to treating patients with type 2 diabetes and act by inhibiting the sodium-glucose co-transporter 2 (SGLT2) on the epithelial cells of the proximal convoluted tubule of the kidneys. Cardiovascular diseases (CVDs) are a major challenge in the management of type 2 diabetes mellitus, and Gliflozins have shown to improve cardiovascular and renal outcomes in subjects with type 2 diabetes presenting cardiovascular diseases. The mechanism by which cardiovascular protection is attributed to gliflozins remains unknown, although there is some research about a hypothetical effect in the myocardial cells. Although platelets are essential for human homeostasis the cells fragments are involved in the pathophysiology of cardiovascular-related diseases. Therefore, the aim of this study is to carry out a pharmacological characterization of the gliflozins (empagliflozin, canagliflozin and dapagliflozin) available on the market in isolated platelets from helathy volunteers. All the experimental protocols were approved by the Ethic Committee from UNICAMP (Number 2.412.312). Our preliminary results showed that markedly inhibited platelet aggregation-challenged by collagen and ADP, although to a lesser extent, also reduced platelet aggregation challenged by U-46619 and thrombin. Moreover, the addition of low concentrations of endothelial mediators potentiated the effects of gliflozins on platelet inhibition.

Key words: Platelets, Empagliflozin, Dapagliflozin, Canagliflozin, Aggregation

Introduction
Gliflozins are a new pharmacological class approved to treating patients with type 2 diabetes and act by inhibiting the transporter named sodium-glucose co-transporter 2 (SGLT2) expressed on the proximal convoluted tubule (1). To date three gliflozins named empagliflozin, dapagliflozin and Canagliflozin were approved by the Brazilian regulatory agency to be used in association with metformin for the treatment of diabetes type 2. In a study with patients with type 2 diabetes and at high risk for cardiovascular events who were receiving standard care, the effects of Empagliflozin (EMPA) were analyzed. Besides the benefits in lowering glucose levels, EMPA has had positive effects in reducing death from cardiovascular causes (38% relative risk reduction) and hospitalization for heart failure (35% relative risk reduction)(2). Because platelets are involved on thrombus formation and EMPA reduced the risk of combined endpoint of hospitalization for heart failure or cardiovascular death our hypothesis is that gliflozins will reduce platelet reactivity.

Results and Discussion
Platelet aggregation was performed with an optical aggregometer at 37 °C with 200 µL of washed platelets placed in glass cuvettes containing a disposable stir bar for constant stirring. Platelet aggregation was carried out in collagen (2 µg/mL), ADP (30 µM), U-46619 (2 µM) and thrombin (0.1 U/mL)-stimulated platelets in the absence and in the presence of the iloprost (0.0 1 nM) and sodium nitroprusside (1 nM). The first objective of the present study was to determine whether gliflozins exerted any dose-dependent inhibition to platelet aggregation triggered by different platelet agonists. As such, gliflozins more effectively inhibited platelet aggregation induced by collagen and ADP, and more effectively inhibited platelet aggregation in the presence of endothelial mediators.

Figure 1. Effects of gliflozins on platelets aggregation. Washed platelets were pre-incubated with canagliflozin, dapagliflozin or empagliflozin (1-100 µM) followed stimulation with different agonists in the (A) absence and in the (B) presence of the iloprost (ILO=0.01 nM) and sodium nitroprusside (SNP-1 nM). N=6. Data represent the mean values ± S.E.M. *P <0.05 compared with vehicle values.

Conclusions
In conclusion, endothelial mediators potentiate the inhibitory effects of gliflozins in aggregation. Altogether, dapagliflozin appears as a promising candidate for further evaluation, and more advanced studies.

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