RESEARCH LETTER

Dapagliflozin reduces plasma concentration of plasminogen activator inhibitor-1 in patients with heart failure with preserved ejection fraction and type 2 diabetes

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Introduction Patients with type 2 diabetes mellitus (DM) have an increased risk of cardiovascular morbidity and mortality due to accelerated development of atherosclerosis.[1-3] Therefore, glucose-lowering therapies with cardioprotective effects are beneficial in pharmacotherapy of these patients. Among glucose-lowering therapies, sodium-glucose cotransporter 2 (SGLT2) inhibitors have been shown to reduce glycemia and to have beneficial effects on atherosclerosis, obesity, insulin resistance, renal function, and blood pressure.[4] More importantly, randomized controlled trials confirmed a reduced risk of worsening heart failure (HF) and cardiovascular death in patients treated with SGLT2 inhibitors (dapagliflozin, empagliflozin) regardless of their diabetic status.[4,5] These clinical benefits might be partially explained by a reduction in thrombin generation and platelet activation related to dapagliflozin use observed in mice models.[6] In addition, empagliflozin decreased plasma concentration of plasminogen activator inhibitor-1 (PAI-1) in patients with type 2 DM.[7] Whether another SGLT2 inhibitor, dapagliflozin, is associated with similar improvement in fibrinolysis is less clear.[8] Therefore, we sought to assess the impact of dapagliflozin treatment on PAI-1 levels in diabetic patients with HF.

Patients and methods Patients with type 2 DM admitted to the outpatient department of the State Hospital in Kielce, Poland, were enrolled in the study. The local bioethics committee at the Jan Kochanowski University, Kielce, Poland approved the study (41/2022). All patients provided their written informed consent. The inclusion criteria were: type 2 DM on oral medications (100% on metformin), age above 18 years, and HF with preserved ejection fraction according to the European Society of Cardiology 2021 guidelines. The exclusion criteria were: contraindications to or prior use of SGLT2 inhibitors, predicted poor compliance, previous or active cancer and / or liver disease, estimated glomerular filtration rate below 60 ml/min/1.73 m^2, pregnancy, current psychiatric treatment, prior acute coronary syndrome, and / or percutaneous coronary intervention / coronary artery bypass grafting. The patients were prescribed 10 mg of dapagliflozin once daily, besides their standard treatment. The primary indication to start dapagliflozin was the treatment of type 2 DM.

Laboratory investigations Fasting venous blood was drawn from the antecubital vein between 7:00 and 10:00 AM. Citrated blood (9:1 of 0.106 M sodium citrate) was centrifuged at 2500 g for 20 minutes at 20°C, while the blood drawn into serum tubes was centrifuged at 1600 g for 10 minutes at 4°C. All blood samples were stored at –80°C until analysis. At baseline and after 60 days of the treatment, routine laboratory assays were used to determine glucose, glycated hemoglobin (HbA_{1c}), and lipid profile. Fibrinolytic proteins in plasma, including PAI-1 antigen, thrombin activatable fibrinolysis inhibitor

TABLE 1 Plasma capacity of fibrinolysis and thrombin generation at baseline and after 60 days of dapagliflozin treatment

Variable	Baseline	Follow-up	Δ	*P* value
CLT, min	120 (99–208)	94 (87–114)	–19.5 (–60 to –10.5)	0.002
PAI-1, ng/ml	22.2 (13.1)	12.9 (5)	–9.4 (9.4)	0.001
TAFI, %	88.6 (13)	93.2 (11.2)	4.6 (20.2)	0.20
tPA, ng/ml	6.3 (2.4)	7.6 (1.9)	1.3 (0.9)	0.32
Lagtime, min	3.3 (0.8)	2.9 (0.8)	–0.4 (0.9)	0.18
ETP, nM*min	1819.6 (326.3)	1625.3 (320.1)	–194.4 (370.2)	0.27
Peak, nM	320.3 (96.8)	320.7 (110.9)	0.4 (131.3)	0.52
ttPeak, min	6.3 (5.8–6.9)	6.6 (4.6–7)	–0.4 (–1.3 to 0.7)	0.39

Data are presented as mean (SD) or median (interquartile range)

Abbreviations: CLT, clot lysis time; ETP, endogenous thrombin potential; PAI-1, plasminogen activator inhibitor-1; TAFI, thrombin activatable fibrinolysis inhibitor; tPA, tissue plasminogen activator; ttPeak, time to peak

(TAFI) antigen, and tissue plasminogen activator (tPA) antigen (all from Hyphen-Biomed, Neuville-sur-Oise, France) were assayed by ELISA according to the manufacturer's instructions. The inter-assay coefficients of variation were below 6%. The blood samples were analyzed in a certified laboratory at the John Paul II hospital, Kraków, Poland.

Clot lysis time Fibrinolysis capacity was determined using a clot lysis time (CLT) assay proposed by the ISTH Subcommittee, as previously described.[9] Briefly, 20 mM calcium chloride, 0.5 U/ml thrombin (Merck, Kenilworth, New Jersey, United States), 15 µM phospholipid vesicles (Rossix, Mölndal, Sweden) and 18 ng/m recombinant tissue plasminogen activator (Actilyse 20 mg, Boehringer Ingelheim, Germany) were mixed with platelet-poor citrated plasma. The mixture was transferred to a microtiter plate, and its turbidity was determined at 405 nm at 37 °C. CLT was defined as the time from the midpoint of clear-to-maximum turbid transition to the midpoint of the maximum-turbid-to-clear transition. The inter-assay coefficients of variation for the lysis variables were below 8%.

Calibrated automated thrombogram Thrombin generation kinetics was measured with the Calibrated Automated Thrombogram (Thrombinoscope BV, Maastricht, the Netherlands) according to the manufacturer's instructions in the 96-well plate fluorometer (Ascent Reader, Thermolabsystems OY, Helsinki, Finland) equipped with a 390/460 filter set at 37 °C.[10] Briefly, 80 µl of plasma were diluted with 20 µl of the PPP-Reagent (Diagnostica Stago, Asnières sur Seine, France) containing about 5 pmol/l recombinant tissue factor, 4 µmol/l phospholipid vesicles, and 20 µl of FluCa solution (Diagnostica Stago). Each plasma sample was analyzed in duplicate, and the intra-assay variability was 6%. The maximum concentration of thrombin formed during the recording time is described as the peak thrombin, and the area under the curve represents endogenous thrombin potential. Lagtime described the initiation phase of coagulation, while time to peak represented the propagation phase of thrombin generation.

Statistical analysis Categorical variables are expressed as number of patients (percentages). Continuous variables are expressed as means with SD or medians (interquartile range). Differences between baseline and follow-up parameters were assessed with the paired *t* test or the Wilcoxon signed-rank test, as appropriate. Correlations were determined by the Pearson and Spearman correlation analysis, as appropriate. All the tests were 2-tailed, and a *P* value below 0.05 was considered significant. All statistical analyses were performed using STATISTICA 13.3 (TIBCO Software Inc., Palo Alto, California, United States).

Results Twelve diabetic patients (mean age 63.5 years, 16.7% women) treated with metformin were enrolled. Of them, 8 had arterial hypertension and 10 had hyperlipidemia treated with statins. Stage 3a chronic kidney disease was noted in 1 patient (Supplementary material, *Table S1*). After 60 days of dapagliflozin treatment, a nonsignificant reduction in HbA_{1c} from mean (SD) of 8 (2.2)% to 7.3 (1.1)% was observed ($P = 0.09$). It was accompanied by a 22% shorter CLT and by 42% reduced PAI-1 plasma concentrations (**TABLE 1**, Supplementary material, *Figure S1*). No differences in tPA levels or TAFI activity were noted after 60 days of dapagliflozin treatment as compared to the baseline. Dapagliflozin did not affect the studied thrombin generation parameters (**TABLE 1**). Of note, there was a strong correlation between on-treatment change (Δ) in CLT and ΔPAI ($r = 0.72$; $P = 0.008$). No significant correlation between ΔCLT or ΔPAI and other clinical and biochemical parameters was observed (data not shown). No change in concomitant medications in the assessed period was required.

Discussion Our study is the first to show that dapagliflozin treatment reduced CLT and PAI-1 level in diabetic patients with HF. In contrast, Sato et al[8] showed that PAI-1 levels only tended to decrease after a 6-month dapagliflozin treatment and ΔPAI-1 in DM patients with coronary artery disease treated with dapagliflozin was similar to ΔPAI-1 in patients treated with conventional drugs. On the other hand, a reduction of PAI-1 by 25% after 12 weeks of treatment with another SGLT2 inhibitor, empagliflozin, was shown by Sakurai et al[7] in patients with type 2 DM.

PAI is a major inhibitor of the fibrinolytic system. More importantly, an increase in PAI-1 activity may lead to hypofibrinolysis, which was shown to be associated with a higher risk of cardiovascular events.[11,12] Hypofibrinolysis and increased PAI-1 level are more common in patients with than without type 2 DM.[11] Moreover, several factors, including hyperglycemia, insulin resistance, dyslipidemia, and visceral obesity, have been suggested as the potential causes of an increased PAI-1 level in diabetic patients.[7] Therefore, a strategy of promoting fibrinolysis via reduction of PAI-1 levels by SGLT2 inhibitors seems an attractive therapeutic option in patients with type 2 DM to reduce their cardiovascular risk.

The mechanisms responsible for the reduction of plasma PAI-1 levels by dapagliflozin and empagliflozin are not clear. The plasma pool of PAI-1 reflects also its production by adipose tissue. Thus, a possible explanation for reducing PAI-1 level by SGLT2 inhibitors is weight loss secondary to glucosuria-induced energy loss. In the study of Sakurai et al,[7] empagliflozin-induced weight loss (mainly visceral adipose tissue loss) positively correlated with changes in plasma PAI-1 level. Another study has shown that plasma PAI-1 concentration decreased following weight reduction related to intensive lifestyle intervention in patients with impaired glucose tolerance.[13] In addition, thrombotic propensity observed in obese patients might be related to the release of adipokines / inflammatory mediators, endothelial dysfunction, enhanced platelet activity, and induced liver production of coagulation factors observed in these patients.[14] Therefore, weight reduction achieved with SGLT2 inhibitors may lead to a more favorable adipokine profile and limit the low-grade inflammation associated with metabolic diseases. However, the reduction in PAI-1 level in our study was observed after just 60 days of dapagliflozin therapy, which is a short period of time to achieve a significant weight loss. On the other hand, a nonsignificant reduction in HbA_{1c} was observed, suggesting an improvement in glycemic control. Chronic hyperglycemia and hyperinsulinemia (insulin resistance) are associated with elevated plasma PAI-1 levels in diabetic patients. Thus, it may suggest an association between better glycemic control and a decrease in PAI-1 level related to dapagliflozin use. Interestingly, such an association was confirmed for other antidiabetic drugs.[15]

Limitations The study's main limitations are small sample size and lack of blood sampling at additional time points. Changes in weight or body mass index, as well as adherence to medications were not assessed during the study. All patients were treated with metformin, and no other oral hypoglycemic agents or insulin were used. Thus, the generalizability of the study's findings may be limited.

Conclusions Short-term treatment with dapagliflozin promoted fibrinolysis and reduced PAI-1 levels in diabetic patients with HF with preserved ejection fraction. This observation may justify the clinical benefits of SGLT2 inhibitors observed in these patients.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

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CONFLICT OF INTEREST None declared.

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