Empagliflozin Ameliorates Leukocyte – Endothelium Cell Interactions and Inflammation in Type 2 Diabetic Patients

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Abstract

Background: SGLT2 inhibitors (iSGLT2) such as empagliflozin can reduce cardiovascular risk in patients with type 2 diabetes, but the underlying molecular mechanisms are yet to be determined. In the present study we evaluate the effects of empagliflozin on anthropometric and endocrine parameters, leukocyte-endothelium interactions, adhesion molecules and NFkB-p65 transcription factor expression.

Methods: Eighteen patients with type 2 diabetes were recruited for the study. Patients received 10 mg/day of empagliflozin according to standard clinical protocols and were followed-up during a 24-week period. Anthropometric and analytical measurements were performed at baseline, 12-weeks and 24-weeks. Interactions between polymorphonuclear leukocytes and human umbilical vein endothelial cells (HUVECs), serum levels of adhesion molecules (P-selectin, VCAM-1 and ICAM-1) and NFkB-p65 protein levels were measured.

Results: We observed a decrease in body weight, BMI and HbA_{1C} levels from 12 weeks of treatment, which had become more pronounced at 24 weeks and was accompanied by a significant reduction in waist circumference, glucose, and hs-CRP levels. Leukocyte-endothelium interactions were reduced due to an enhancement of leukocyte rolling velocity from 12 weeks onwards, together with a significant decrease in leukocyte rolling flux and adhesion at 24 weeks. Accordingly, a significant decrease in ICAM-1 levels and NFkB-p65 expression were observed.

Conclusions: Empagliflozin reduced leukocyte-endothelium interactions, adhesion molecules and NFkB-p65 expression in type 2 diabetic patients after 24 weeks of treatment.

Background

Cardiovascular diseases (CVDs) are the most common cause of mortality in type 2 diabetic patients [1, 2], and hyperglycaemia, hypertension, dyslipidaemia, and obesity are important risk factors for CVDs. Particularly, under chronic hyperglycaemic conditions, elevated levels of circulating advanced glycation end products (AGEs) play a central role in the pathogenesis of the micro- and macrovascular complications related with type 2 diabetes [1, 3], promoting cellular dysfunction and regulating endothelial cell permeability, monocyte migration and expression of adhesion molecules [4–6], such as P-selectin, vascular adhesion molecule-1 (VCAM-1), and the intercellular adhesion molecule-1 (ICAM-1). Another important aspect in the development of CVDs is the atherosclerotic process, which is mediated by leukocytes such as peripheral polymorphonuclear leukocytes (PMNs). PMNs are activated under chronic hyperglycaemia and play a crucial role in CVDs by promoting cellular and endothelial impairment due to vessel recruitment and leukocyte aggregation [7] or through reactive oxygen species (ROS) production, which promotes oxidative stress and NFκB activation, altering, in turn, proinflammatory gene expression and eventually inducing cardiovascular impairment [8].

In addition, the relationship between glycated haemoglobin levels (HbA_{1C}), inflammation and CVDs points to modulation of HbA_{1C} levels as a potentially interesting therapeutic goal [9]. In this sense,

inhibitors of sodium and glucose co-transporter 2 (iSGLT2) are one class among many antidiabetic agents and could represent an effective therapeutic strategy given their safety and potential both as monotherapy and in combination with other anti-diabetic drugs [10]. iSGLT2 acts by directly modulating kidney glucose excretion through the induction of glycosuria with an insulin-independent mechanism of action [10, 11]. Empagliflozin is an iSGLT2 approved for the treatment of adults with type 2 diabetes, and, as demonstrated by the EMPA-REG OUTCOME study, presents both cardioprotective and renoprotective effects [12].

In the present study, we investigate the potential therapeutic benefits of empagliflozin treatment (12 and 24-weeks) on leukocyte-endothelial interactions, adhesion molecules and NFκB-p65 expression, all of which are implicated in the development of CVDs.

Methods

Patients and sample collection

This is an observational and prospective follow-up study of a cohort of eighteen patients diagnosed with type 2 diabetes according to the American Diabetes Association´s criteria [13] attending to the Endocrinology Department of the University Hospital Doctor Peset (Valencia, Spain). Patients were recruited when physicians added empagliflozin to their usual treatment according to standard clinical protocols in the hospital. Two of the eighteen patients were excluded from the study due to lack of adherence to treatment. Subjects were asked to follow a scheduled visit program that included follow-up at 12 and 24-weeks after the first visit.

The inclusion criteria were as follows: age between 40 and 70 years and evolution of diabetes greater than 10 years. The exclusion criteria were as follows: severe diabetic neuropathy, significant renal impairment (creatinine > 1.5 mg/dL or eGFR < 60 mL/min/1.73 m^2), morbid obesity (BMI > 40 kg/m^2), smoking habit or frequent alcohol intake, chronic diseases others than those directly related to cardiovascular risk. To exclude alterations due to other current medication, patients were also excluded if their hypoglycaemic, lipid-lowering, or antihypertensive treatment had been/was altered (changes in dose or type of drug) in the previous year or during the study. Empagliflozin was administered orally at doses of 10 mg/day according to normal clinical practice [14, 15]. Measurements were assessed at baseline and at 12 and 24-weeks of empagliflozin treatment. The most common side effects for iSGLT2 treatment described in literature are genital and urinary tract infections, but none of our subjects developed any of these conditions during the study.

All subjects were informed about the study procedures and gave their informed written consent. The study was performed in compliance with the statement of ethical principles for medical research of the Declaration of Helsinki and obtained the approval from the Hospital’s Ethics Committee (CEIC 30/17).

Anthropometric and biochemical analysis

During the first and follow-up appointments, patients underwent a physical examination to determine the following anthropometrical parameters: weight (kg), height (m), waist circumference (cm), and systolic (SBP) and diastolic blood pressure (DBP, mmHG). After 12 h of overnight fasting, blood samples were taken from 8:00 to 10:00 a.m. and centrifuged (1.500 g, 10 min, 4 °C) to separate serum or plasma prior to determining biochemical and molecular parameters. Biochemical determinations were carried out by our Hospital´s Clinical Analysis Service and evaluated as usual [16]: glucose, triglycerides and total cholesterol levels in serum were measured by means of an enzymatic method; insulin levels were calculated by immunochemiluminescence and insulin resistance by homeostasis model assessment (HOMA-IR = [fasting insulin (μU/ml) x fasting glucose (mg/dl)]/405); percentage of HbA_{1C} was measured with an automated glycohaemoglobin analyzer (Arkray Inc., Kyoto, Japan); levels of high-density lipoprotein cholesterol (HDL-c) were assessed with a Beckman LX-20 autoanalyzer (Beckman Coulter, La Brea, CA, US); low-density lipoprotein cholesterol (LDL-c) was estimated with Friedewald's formula; and high-sensitive C-reactive protein (hs-CRP) levels were determined by an immunonephelometric assay (Behring Nephelometer II, Dade Behring, Inc., Newark, DE, USA).

Leukocyte isolation

Citrated blood samples were incubated for 45 min with 3% w/v dextran in phosphate-buffered saline solution (PBS; Sigma Aldrich, St. Louis, MO, USA). For PMNs isolation, supernatants were placed on Ficoll-Hypaque (GE Healthcare, Barcelona, Spain) and gradient centrifugation was performed (650 g for 25 min at RT). The supernatant was discarded, and the bottom phase containing PMNs pellet was incubated for 5 min at RT with lysis buffer in order to eliminate the remaining erythrocytes. The sample was then centrifuged (1.200 rpm, 5 min) and washed twice with Hank´s Balance Salt Solution (HBSS; Sigma Aldrich, St. Louis, MO, USA). Finally, the pellet was resuspended in complete RPMI medium (Biowest-bw, Nuaillé, France) supplemented with 10% FBS. Aliquots of 1.2×10^6 cell/mL were employed in the subsequent experiments.

Leukocyte-endothelium interactions and cellular adhesion molecules evaluation

For adhesion assays we used an *ex vivo* model based on a parallel plate flow chamber, as described before [17]. In brief, human umbilical vein endothelial cells (HUVECs) were harvested from fresh umbilical cords obtained from healthy donors by means of collagenase solution (1 mg/mL in PBS; Sigma Aldrich, St. Louis, MO, USA) digestion for 17 min. Primary cultures of HUVECs were grown over fibronectin-coated cell culture dishes (Corning, NY, USA) and incubated with complete Endothelial Cell Basal Medium-2 supplemented with Growth Medium-2 Supplement kit (both from PromoCell GmbH, Heidelberg, Germany) until HUVECs reach confluence. A portion of 5 × 25 mm of the HUVECs monolayer was exposed to the PMN flux and recorded using an inverted microscope (Nikon Eclipse TE 2000-S, Amstelveen, The Netherlands) coupled to a video camera (Sony Exware HAD, Koeln, Germany). Along the HUVECs monolayer, suspensions of PMNs were perfused at flow rate of 0.36 mL/min (human blood flow rate in

physiological condition). Real time images of the flow-exposed monolayer were recorded for 5 min and further analyzed to extrapolate leukocyte rolling flux, rolling velocity and adhesion [17].

Levels of cellular adhesion molecules in serum samples (P-selectin, ICAM-1 and VCAM-1) were evaluated with a Milliplex Multiplex Assay Kit (Millipore Corporation, Billerica, MA, USA) in a Luminex 200 flow analyzer system (Luminex Corp., Austin, TX, USA) according to the manufacturer`s instructions. The intra-serial CV was < 5.0%, and the inter-serial CV was < 15.0%, for all determinations.

Western Blot analysis

Leukocytes were incubated for 15 min on ice with a lysis buffer (400 mM NaCl, 20 mM HEPES pH 7.5, 0.1 mM EDTA, 20% glycerol, 10 µM Na2MoO4 and 0.5% Nonidet P-40) containing protease inhibitors (10 mM β-glycerolphosphate, 10 mM NaF, 10 mM PNP and 1 mM Na_3VO_4) and dithiothreitol 1 mM and were then centrifuged at 4 °C for 15 min. Protein concentrations were determined using the BCA protein assay kit (Thermo Fisher Scientific, IL, US). Protein samples (25 µg) were resolved by means of sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to nitrocellulose membranes. After blocking, they were incubated with primary antibodies overnight at 4 °C. We used the following primary antibodies: anti-NFκB-p65 (phospho S536) rabbit polyclonal antibody (Abcam, Cambridge, MA) and anti-β actin rabbit polyclonal antibody (Sigma Aldrich, Missouri, US). Blots were incubated with goat anti-rabbit HRP secondary antibody (Millipore Iberica, Madrid, Spain) and developed for 2 min with supersignal west femto (Thermo Fisher Scientific, IL, US). Chemiluminescence signals were detected with a Fusion FX5 acquisition system (Vilbert Lourmat, Marne La Vallée, France) and analyzed by densitometry using Bio1D software (Vilbert Lourmat, Marne La Vallée, France). Protein bands were normalized to the expression of actin in the same sample.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 7.00 (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). Parametric data were expressed as mean ± standard deviation (SD) and non-parametric data as a median with 25th and 75th percentiles. Statistical significance between groups was assessed by one-way ANOVA followed by a Tukey post-hoc test for parametric data or the Friedman test followed by Dunn's multiple comparisons test for non-parametric data. A paired t test was employed when two groups were compared. Differences of $p < 0.05$ were considered statistically significant. Bar graphs show mean ± standard error of the mean (SEM).

Results

Anthropometric and biochemical analysis

This study initially involved eighteen type 2 diabetic patients who initiated treatment with empagliflozin. All patients had received stable glucose-lowering therapy for at least 12 months before being recruited for

the study, and they continued with this therapy in combination with empagliflozin during the entire study. Additionally, fourteen patients were taking statins as lipid-lowering therapy, nine patients were on antihypertensive medication, and six were receiving diuretic treatment. In terms of medical history of microvascular complications, four and five diabetic patients were affected by retinopathy and nephropathy, respectively.

Table 1 shows the anthropometric and biochemical data for our study population. We observed that empagliflozin significantly reduced body weight at 12-weeks of treatment ($p < 0.01$) and that this reduction was maintained at 24 weeks ($p < 0.01$ vs. baseline and $p < 0.05$ vs. 12-weeks). In parallel, a significant waist circumference reduction was observed at 24-weeks ($p < 0.01$ vs baseline and $p < 0.05$ vs. 12-weeks). These data were confirmed by a progressive reduction in BMI ($p < 0.05$ at 12-weeks and $p < 0.01$ at 24 weeks, both vs. baseline). Data showed a decrease in glucose levels after 24-weeks ($p < 0.05$) and in HbA_{1C} levels from 12-weeks onwards ($p < 0.05$). HOMA-IR decreased throughout the treatment, almost reaching significance at 24-weeks ($p = 0.063$).

Total cholesterol and triglycerides increased significantly at 12-weeks ($p < 0.05$) and were reduced at 24-weeks, though this decrease was significant only in triglycerides ($p < 0.05$ vs. 12-weeks). No significant changes in LDL-c and HDL-c were observed after treatment with empagliflozin. Patients receiving insulin as part of their treatment were excluded from HOMA-IR and insulin assessments. Lastly, we observed an increase in hs-CRP levels at 12-weeks of treatment, though a decrease was detected at 24 weeks ($p < 0.05$ vs. 12-weeks).

Table 1
Anthropometric characteristics of the study population at baseline, at 12 and 24-week follow-up.

	Baseline	12-week empagliflozin	24-week empagliflozin
N	16	16	16
Age (years)	59.7 ± 10.8	-	-
Weight (kg)	85.7 ± 20.1	82.9 ± 20.3**	81.6 ± 20.3** #
Waist circumference (cm)	102.7 ± 12.3	99.8 ± 13.5	97.1 ± 13.7** #
BMI	31.4 ± 5.3	30.3 ± 5.4*	29.9 ± 5.6**
SBP (mmHg)	139.5 ± 26.9	139.6 ± 24.6	133.9 ± 21.7
DBP (mmHg)	76.4 ± 14.1	81.0 ± 16.3	73.9 ± 12.7
Glucose (mg/dL)	149.1 ± 35.9	134.1 ± 32.6	125.2 ± 19.9*
HbA_{1C} (%)	7.6 ± 1.3	7.2 ± 1.3*	6.8 ± 0.9*
Insulin (μUI/mL)	9.6 ± 5.4	9.6 ± 5.5	9.5 ± 5.9
HOMA-IR	3.88 ± 2.16	3.32 ± 1.54	3.10 ± 2.10
Total cholesterol (mg/dL)	141.0 ± 25.4	154.7 ± 27.6*	149.5 ± 27.1
LDL-c (mg/dL)	82.3 ± 16.9	87.2 ± 16.6	89.1 ± 19.8
HDL-c (mg/dL)	46.1 ± 6.1	43.2 ± 7.1	47.5 ± 3.7
Triglycerides (mg/dL)	95 (86–128)	119.0 (102–206)*	111 (87–130)#
hs-CRP	3.19 ± 3.48	4.36 ± 4.00	3.17 ± 2.75#

Data are expressed as mean ± SD for parametric variables and as median (interquartile range) for non-parametric data. The following statistical analyses were performed: for parametric variables, a repeated measures one-way ANOVA followed by Tukey´s multiple comparisons test; for non-parametric variables, a Friedman test followed by Dunn´s multiple comparisons test. *$p < 0.05$ vs. baseline, **$p < 0.01$ vs. baseline, #$p < 0.05$ vs. 12-week empagliflozin treatment. Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HbA_{1C}, glycated hemoglobin A_{1C}; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high sensitive C-reactive protein; LDL-c, low-density lipoprotein cholesterol; SBP, systolic blood pressure.

Leukocyte-endothelium interactions and adhesion molecules expression

Leukocyte rolling velocity (Fig. 1A) was enhanced at 12 and 24-weeks of treatment with empagliflozin ($p < 0.05$ and $p < 0.01$, respectively) compared to baseline. Regarding PMNs rolling flux and adhesion (Fig. 1B and C), both data showed a tendency to decrease, which became significant at 24-weeks of treatment ($p < 0.05$ both).

To explore in more depth, the results obtained during the leukocyte-endothelium interactions assays, we studied the expression of adhesion molecules in serum at 24-weeks of treatment. We observed a significant reduction in ICAM-1 expression levels at 24-weeks (Fig. 2C; $p < 0.05$), but not in the expression of P-selectin and VCAM-1 (Fig. 2A and 2B).

Levels of NFκB-p65

Changes in proinflammatory protein expression, measured in terms of NFκB-p65, were evaluated to clarify the mechanisms involved in the amelioration of leukocyte-endothelium interactions after empagliflozin treatment. In this context, a higher peak in NFκB-p65 levels was observed in leukocytes from type 2 diabetic patients at baseline, which was significantly decreased at 24-weeks of empagliflozin treatment ($p < 0.05$, Fig. 3).

Discussion

In this observational, prospective follow-up study we have analysed the effects of the iSGLT2 empagliflozin on leukocyte-endothelium interactions, adhesion molecules and NFκB-p65 expression after 12 and 24-weeks of treatment. In addition, we have explored some of the beneficial effects of empagliflozin, including weight reduction [18], decrease in BMI and waist circumference, and improvement in metabolic and lipid profile [10] (glucose, HbA_{1C}, total cholesterol and triglycerides).

We have evaluated the effects of empaglifozin on leukocyte-endothelial cell interactions by using an *ex vivo* parallel-flow chamber assay which mimics physiological blood flow [16]. As a result, we have observed that empagliflozin increases PMNs rolling velocity in consonance with a decrease in PMN rolling flux and adhesion at 24-weeks of treatment. These actions result in a decrease in leukocyte-endothelium interactions, suggesting that this drug exerts a beneficial effect by protecting against the early stages of atherosclerotic process. Hyperglycaemia and increased levels of HbA_{1C} are key factors in the atherosclerosis process, and are related to enhanced leukocyte-endothelium interactions, mitochondrial impairment, and oxidative stress [15, 19]. Enhanced leukocyte-endothelium interactions have also been linked to insulin resistance [20–22]. Our data confirm that empagliflozin treatment reduces HOMA-IR, as described previously [23], which would improve insulin resistance. In line with a beneficial effect of empagliflozin, the drug has recently been reported to protect the heart from

inflammation and energy depletion via AMPK activation [24], as well as preventing doxorubicin-induced myocardial dysfunction [25].

Endothelial-leukocyte interactions depend heavily on the levels of adhesion molecules, resulting from vascular inflammation and dysfunction and involved in the recruitment of immune cells and platelets to the endothelium. In this sense, the present results show a reduction in the expression of the adhesion molecule ICAM-1 and the inflammatory marker hs-CRP after 24-weeks of empagliflozin treatment. These results are in accordance with those previously reported by our group showing that empagliflozin treatment reduced levels of the inflammatory enzyme myeloperoxidase - actively involved in the development of microvascular alterations [26] - and increased release of the anti-inflammatory interleukin-10 (IL-10) [15]. Considered as a whole, these data suggest that empagliflozin ameliorates the inflammatory state and reduces the risk of CVDs. In support of this, empagliflozin has been shown to reverse obesity and insulin resistance through fat browning and alternative macrophage activation in mice fed a high-fat diet [27], and to reduce inflammation and boost the antioxidant response in leukocytes from type 2 diabetic patients [15].

It is well known that type 2 diabetes is related to oxidative stress and that this leads to pro-inflammatory responses. In fact, enhanced levels of ROS activate the pro-inflammatory nuclear factor NFκB, thus contributing to insulin resistance [28]. Considering this, we decided to explore whether empagliflozin has an effect on NFκB-p65 protein expression in leukocytes from type 2 diabetic patients at 12 and 24-weeks of treatment. When we assessed the expression of p65 (phospho S563) in total cell protein extracts we found a decrease in the protein NFκB-p65 after 24-weeks of treatment. This effect highlights empagliflozin as a molecule with anti-inflammatory properties that modulate, not only oxidative stress and leukocyte-endothelium interactions, but also the inflammatory response. In accordance with these results, *Quariagiello et al*., demonstrated that empagliflozin had cardioprotective and anti-inflammatory effects when administered together with doxorubicin in a mouse model, by decreasing the expression of leukotriene B4 and NFκB and that of IL-1β, IL-6 and IL-8 [29]. In this context, it should be mentioned that other studies have not demonstrated any change in NFκB; for example, *Uthman et al.* reported that empagliflozin and dapagliflozin reduced ROS generation and restored nitric oxide (NO) bioavailability in TNF α-stimulated human coronary arterial endothelial cells, but did not have an effect on NFκB expression.

Conclusions

In conclusion, the present study provides evidence that treatment with empagliflozin decreases leukocyte-endothelium interactions, adhesion molecules and NFκB expression in type 2 diabetes. This highlights the value of this drug for preventing the atherosclerotic process, inflammation and, consequently, possible cardiovascular events in type 2 diabetic patients.

List Of Abbreviations

ADP: adenosine diphosphate

AGEs: advanced glycosylation-end products

AMPK: AMP-activated protein kinase

BMI: body mass index

CV: coefficient of variation

CVDs: cardiovascular disease

DBP: diastolic blood pressure

FBS: fetal bovine serum

HbA_{1C}: glycated hemoglobin A_{1C}

HBSS: Hank's Balanced Salt Solution

HDL-c: high-density lipoprotein cholesterol

HOMA-IR: homeostatic model assessment for insulin resistance

hs-CRP: high sensitive C-reactive protein

HUVEC: human umbilical vein endothelial cells

ICAM-1: intercellular adhesion molecule-1

IL-1β, 6, 8 or 10: interleukin 1β, 6, 8, or 10

iSGLT2: Inhibitors of sodium and glucose co-transporter 2

LDL-c: low-density lipoprotein cholesterol

NFκB: Nuclear factor kappa-light-chain-enhancer of activated B cells

NO: nitric oxide

PBS: phosphate-buffered saline solution

PMNs: peripheral polymorphonuclear leukocytes

ROS: reactive oxygen species

RPMI: Roswell Park Memorial Institute

RT: room temperature

SBP: systolic blood pressure

SD: standard deviation

SEM: standard error of the mean

TNFα: tumor necrosis factor alpha

VCAM-1: vascular adhesion molecule-1

Declarations

Ethics approval and consent to participate:

The study was performed in compliance with the ethical principles established in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of the University Hospital Dr. Peset (CEIC 30/17). Written informed consent was obtained from all the participants before they participated in the study.

Consent for publication:

Not applicable

Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests. The funding sponsors had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Authors' contributions:

Conceptualization, V.M.V. and M.R.; Data curation, F.I., F.C., A.M.d.M., S.L.-D., T.V., B.N. and E.S; Formal analysis, F.I., F.C., A.M.d.M., S.L.-D., T.V., B.N. and C.M.; Funding acquisition, V.M.V. and M.R.; Investigation, F.I., F.C., V.M.V. and M.R.; Methodology, V.M.V.; Project administration, V.M.V. and M.R.; Resources, V.M.V. and M.R.; Supervision, V.M.V., and M.R.; Validation, V.M.V., M.R. and C.M.; Visualization, F.I., F.C., A.M.d.M. and T.V.; Writing – original draft, F.I., F.C., V.M.V. and M.R.; Writing – review & editing, F.I., F.C., A.M.d.M., S.L.-D., T.V., B.N., E.S., C.M., M.R. and V.M.V.

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References

1. Strain WD, Paldánius PM. Diabetes, cardiovascular disease and the microcirculation. Cardiovasc Diabetol 2018;17:57. https://doi.org/10.1186/s12933-018-0703-2.
2. Lahnwong S, Chattipakorn SC, Chattipakorn N. Potential mechanisms responsible for cardioprotective effects of sodium-glucose co-transporter 2 inhibitors. Cardiovasc Diabetol 2018;17:101. https://doi.org/10.1186/s12933-018-0745-5.
3. Stirban A, Gawlowski T, Roden M. Vascular effects of advanced glycation endproducts: Clinical effects and molecular mechanisms. Mol Metab 2014;3:94–108. https://doi.org/10.1016/j.molmet.2013.11.006.
4. Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. Immunity 2010;32:593–604. https://doi.org/10.1016/j.immuni.2010.05.007.
5. Bevilacqua MP, Stengelin S, Gimbrone MA, Seed B. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. Science 1989;243:1160–5. https://doi.org/10.1126/science.2466335.
6. Cid MC, Cebrián M, Font C, Coll-Vinent B, Hernández-Rodríguez J, Esparza J, et al. Cell adhesion molecules in the development of inflammatory infiltrates in giant cell arteritis: inflammation-induced angiogenesis as the preferential site of leukocyte-endothelial cell interactions. Arthritis Rheum 2000;43:184–94. https://doi.org/10.1002/1529-0131(200001)43:1<184::AID-ANR23>3.0.CO;2-N.

7. Jay D, Hitomi H, Griendling KK. Oxidative stress and diabetic cardiovascular complications. Free Radic Biol Med 2006;40:183–92. https://doi.org/10.1016/j.freeradbiomed.2005.06.018.
8. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813–20. https://doi.org/10.1038/414813a.
9. Goff DC, Gerstein HC, Ginsberg HN, Cushman WC, Margolis KL, Byington RP, et al. Prevention of cardiovascular disease in persons with type 2 diabetes mellitus: current knowledge and rationale for the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. Am J Cardiol 2007;99:4i–20i. https://doi.org/10.1016/j.amjcard.2007.03.002.
10. Frampton JE. Empagliflozin: A Review in Type 2 Diabetes. Drugs 2018;78:1037–48. https://doi.org/10.1007/s40265-018-0937-z.
11. Abdul-Ghani M, Del Prato S, Chilton R, DeFronzo RA. SGLT2 Inhibitors and Cardiovascular Risk: Lessons Learned From the EMPA-REG OUTCOME Study. Diabetes Care 2016;39:717–25. https://doi.org/10.2337/dc16-0041.
12. Schernthaner G, Schernthaner-Reiter MH, Schernthaner G-H. EMPA-REG and Other Cardiovascular Outcome Trials of Glucose-lowering Agents: Implications for Future Treatment Strategies in Type 2 Diabetes Mellitus. Clin Ther 2016;38:1288–98. https://doi.org/10.1016/j.clinthera.2016.04.037.
13. American Diabetes Association. 2. Classification and Diagnosis of Diabetes. Diabetes Care 2015;38:S8–16. https://doi.org/10.2337/dc15-S005.
14. Safety and Efficacy Study of Empagliflozin and Metformin for 24 Weeks in Treatment Naive Patients With Type 2 Diabetes - Study Results - ClinicalTrials.gov n.d. https://clinicaltrials.gov/ct2/show/results/NCT01719003 (accessed February 25, 2020).
15. Iannantuoni F, M de Marañon A, Diaz-Morales N, Falcon R, Bañuls C, Abad-Jimenez Z, et al. The SGLT2 Inhibitor Empagliflozin Ameliorates the Inflammatory Profile in Type 2 Diabetic Patients and Promotes an Antioxidant Response in Leukocytes. J Clin Med 2019;8. https://doi.org/10.3390/jcm8111814.
16. Escribano-Lopez I, Diaz-Morales N, Iannantuoni F, Lopez-Domenech S, de Marañon AM, Abad-Jimenez Z, et al. The mitochondrial antioxidant SS-31 increases SIRT1 levels and ameliorates inflammation, oxidative stress and leukocyte-endothelium interactions in type 2 diabetes. Sci Rep 2018;8:15862. https://doi.org/10.1038/s41598-018-34251-8.
17. Bañuls C, de Marañon AM, Veses S, Castro-Vega I, López-Domènech S, Salom-Vendrell C, et al. Malnutrition impairs mitochondrial function and leukocyte activation. Nutrition Journal 2019;18:89. https://doi.org/10.1186/s12937-019-0514-7.
18. Ferrannini G, Hach T, Crowe S, Sanghvi A, Hall KD, Ferrannini E. Energy Balance After Sodium–Glucose Cotransporter 2 Inhibition. Diabetes Care 2015;38:1730–5. https://doi.org/10.2337/dc15-0355.
19. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 2000;404:787–90. https://doi.org/10.1038/35008121.

20. Burgos-Morón E, Abad-Jiménez Z, Martínez de Marañón A, Iannantuoni F, Escribano-López I, López-Domènech S, et al. Relationship between Oxidative Stress, ER Stress, and Inflammation in Type 2 Diabetes: The Battle Continues. Journal of Clinical Medicine 2019;8:1385. https://doi.org/10.3390/jcm8091385.
21. Nigro J, Osman N, Dart AM, Little PJ. Insulin resistance and atherosclerosis. Endocr Rev 2006;27:242–59. https://doi.org/10.1210/er.2005-0007.
22. Pansuria M, Xi H, Li L, Yang X-F, Wang H. Insulin resistance, metabolic stress, and atherosclerosis. Front Biosci (Schol Ed) 2012;4:916–31. https://doi.org/10.2741/s308.
23. Hattori S. Anti-inflammatory effects of empagliflozin in patients with type 2 diabetes and insulin resistance. Diabetol Metab Syndr 2018;10:93. https://doi.org/10.1186/s13098-018-0395-5.
24. Koyani CN, Plastira I, Sourij H, Hallström S, Schmidt A, Rainer PP, et al. Empagliflozin protects heart from inflammation and energy depletion via AMPK activation. Pharmacol Res 2020;158:104870. https://doi.org/10.1016/j.phrs.2020.104870.
25. Sabatino J, De Rosa S, Tammè L, Iaconetti C, Sorrentino S, Polimeni A, et al. Empagliflozin prevents doxorubicin-induced myocardial dysfunction. Cardiovasc Diabetol 2020;19. https://doi.org/10.1186/s12933-020-01040-5.
26. Ndrepepa G. Myeloperoxidase - A bridge linking inflammation and oxidative stress with cardiovascular disease. Clin Chim Acta 2019;493:36–51. https://doi.org/10.1016/j.cca.2019.02.022.
27. Xu L, Nagata N, Chen G, Nagashimada M, Zhuge F, Ni Y, et al. Empagliflozin reverses obesity and insulin resistance through fat browning and alternative macrophage activation in mice fed a high-fat diet. BMJ Open Diabetes Res Care 2019;7:e000783. https://doi.org/10.1136/bmjdrc-2019-000783.
28. Rains JL, Jain SK. OXIDATIVE STRESS, INSULIN SIGNALING AND DIABETES. Free Radic Biol Med 2011;50:567–75. https://doi.org/10.1016/j.freeradbiomed.2010.12.006.
29. Quagliariello V, Coppola C, Rea D, Maurea C, Barbieri A, Botti G, et al. 1894P - Cardioprotective and anti-inflammatory effects of empagliflozin during treatment with doxorubicin: A cellular and preclinical study. Annals of Oncology 2019;30:v768. https://doi.org/10.1093/annonc/mdz268.021.

Figures

Figure 1

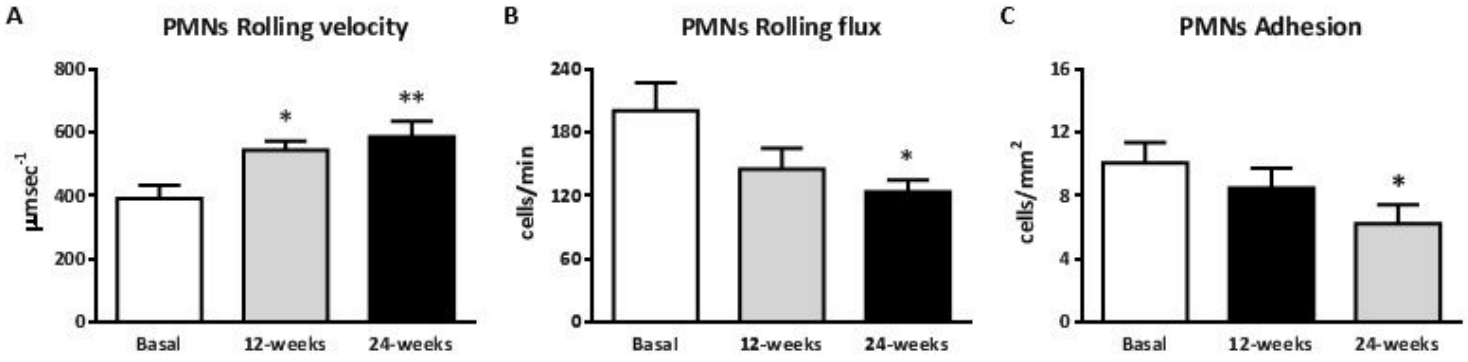

Figure 1

Effects of empagliflozin treatment in leukocyte-endothelium interactions in type 2 diabetic patients at basal, 12 and 24-weeks of treatment. (A) Leukocyte rolling velocity (μm*sec-1), (B) Rolling flux (cells/min), (C) Leukocyte adhesion (cells/mm2). Data are expressed as mean + SEM. *p<0.05 and **p<0.01 vs baseline. Abbreviations: PMN, polymorphonuclear leukocytes.

Figure 2

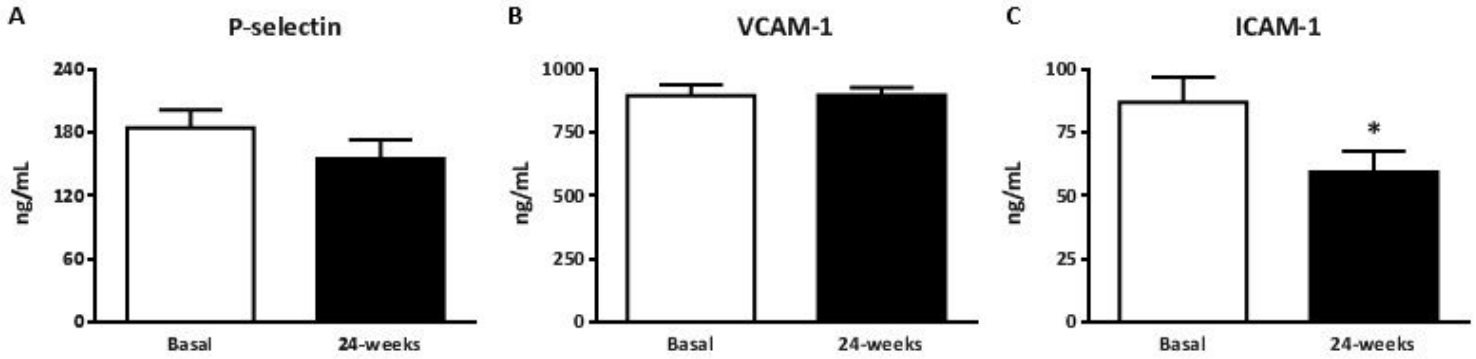

Figure 2

Effects of empagliflozin treatment in serum soluble cell adhesion molecules in type 2 diabetic patients at basal, 12 and 24-weeks of treatment. (A) P-selectin levels, (B) VCAM-1 levels and (C) ICAM-1 levels. Data are expressed as mean + SEM. *p<0.05 vs baseline. Abbreviations: ICAM-1: intercellular adhesion molecule-1; P-selectin: platelet selectin; VCAM-1: vascular cell adhesion molecule-1.

Figure 3

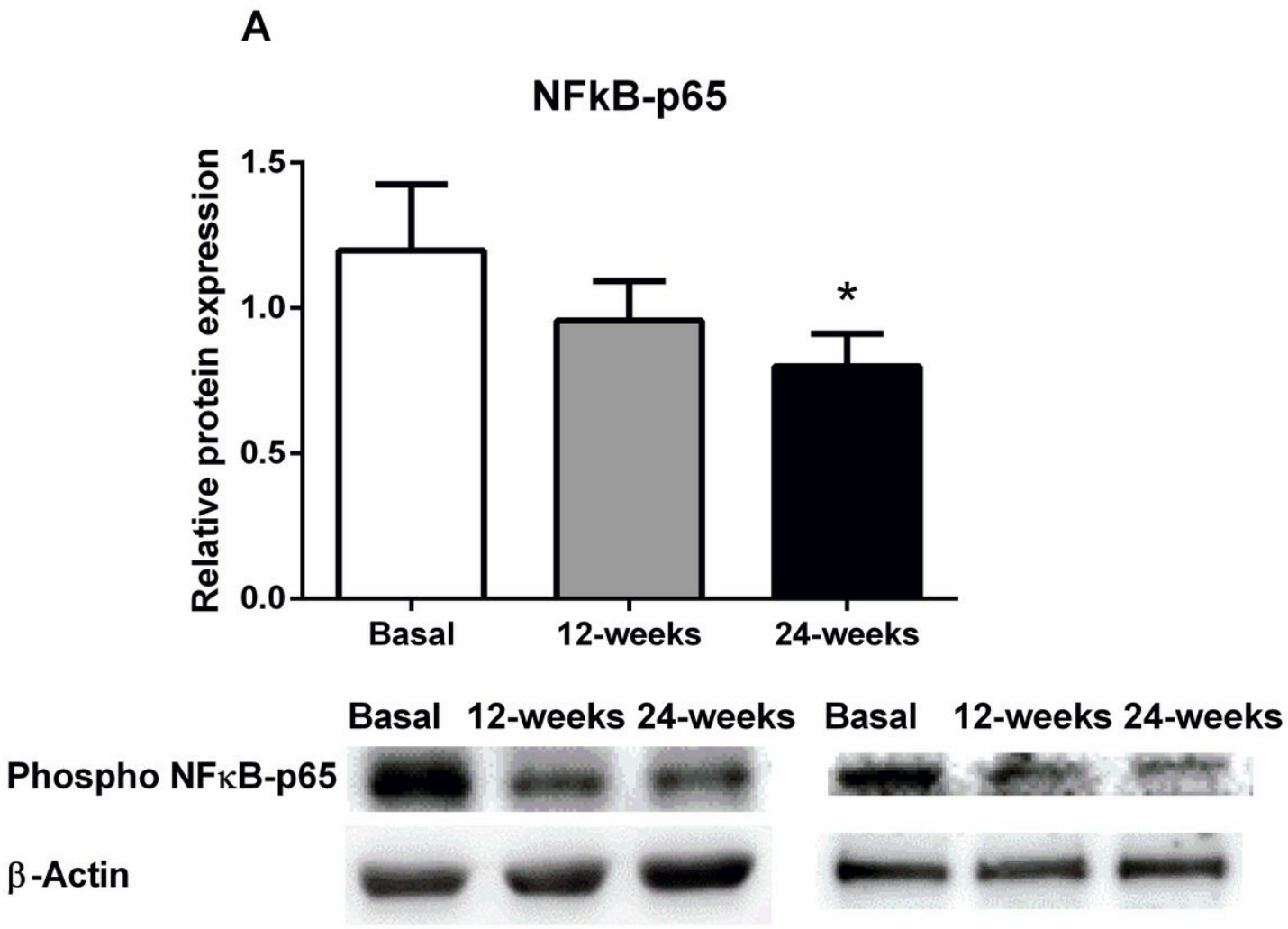

Figure 3

Effects of empagliflozin treatment on the expression of NFκB-p65 in leukocytes of type 2 diabetic patients at basal, 12 and 24-weeks of treatment. Protein levels of NFκB-p65 and representative WB images. Data are expressed as mean + SEM. *p<0.05 vs baseline.