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The SGLT2 inhibitor empagliflozin improves insulin sensitivity in *db/db* mice both as monotherapy and in combination with linagliptin

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ABSTRACT

Aims. Combining different drug classes to improve glycemic control is one treatment strategy for type 2 diabetes. The effects on insulin sensitivity of long-term treatment with the sodium glucose co-transporter 2 (SGLT2) inhibitor empagliflozin alone or co-administered with the dipeptidyl peptidase-4 inhibitor linagliptin (both approved antidiabetes drugs) were investigated in mice using euglycemic–hyperinsulinemic clamps.

Materials and Methods. *db/db* mice (n = 15/group) were treated for 8 weeks with 10 mg/kg/day empagliflozin monotherapy, 10 mg/kg/day empagliflozin plus 3 mg/kg/day linagliptin combination therapy, or 3 mg/kg/day linagliptin monotherapy. At the end of the study, euglycemic–hyperinsulinemic clamp studies were performed 4 days after the last dose of treatment.

Results. HbA1c and 2-hour fasting glucose concentrations were improved with empagliflozin monotherapy and combination therapy compared with vehicle and linagliptin monotherapy. During the clamp, glucose disposal rates increased and hepatic glucose production decreased with empagliflozin monotherapy and combination therapy compared with vehicle and linagliptin monotherapy. Glucose uptake in liver and kidney was higher with empagliflozin monotherapy and combination therapy compared with vehicle; glucose uptake into both muscle and adipose tissue was only affected by linagliptin treatment. Empagliflozin and combination therapy altered the expression of genes involved in the inflammatory response, fatty acid synthesis and oxidation.

Conclusions. These findings suggest that the insulin-sensitizing effects of SGLT2 inhibition contribute to improvements in glycemic control in insulin-resistant states.

Abbreviations: DIO, diet-induced obese; DPP, dipeptidyl peptidase; FAS, fatty acid synthase; FPG, fasting plasma glucose; GDR, glucose disposal rate; HbA1c, glycated hemoglobin; HGP, hepatic glucose production; HPRT, hypoxanthine phosphoribosyltransferase; OGTT, oral glucose tolerance test; PTP, protein tyrosine phosphatase; SCD, stearoyl-CoA desaturase; SGLT, sodium glucose co-transporter; SOCS, suppressor of cytokine signaling; T2D, type 2 diabetes; ZDF, Zucker diabetic fatty.

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1. Introduction

Empagliflozin, a potent and selective sodium glucose co-transporter 2 (SGLT2) inhibitor [1], was recently approved for the treatment of type 2 diabetes (T2D) [2]. Studies have shown that single doses of empagliflozin result in dose-dependent increases in urinary glucose excretion and decreases in blood glucose levels [3]. Multiple doses of empagliflozin over 5 weeks significantly reduce fasting blood glucose and glycated hemoglobin (HbA1c) levels, and improve insulin sensitivity in Zucker diabetic fatty (ZDF) rats [3]. Empagliflozin also improves glycemic control alone and in combination with insulin in streptozotocin-induced diabetic rats [4].

Linagliptin, a xanthine-based, highly potent and long-acting non-peptidomimetic dipeptidyl peptidase (DPP)-4 inhibitor, is approved for the treatment of T2D [5,6]. In animal and in vitro studies, linagliptin demonstrated a greater inhibition of DPP-4 than alogliptin, saxagliptin, sitagliptin, or vildagliptin [6]. After absorption, linagliptin binds to plasma proteins in a concentration-dependent manner, giving the drug a nonlinear pharmacokinetic profile [7]. Unlike other DPP-4 inhibitors that are cleared by the kidneys, linagliptin is mainly excreted in the feces [8,9]. We have shown that linagliptin dose-dependently improves insulin sensitivity in diet-induced obese (DIO) C57BL/6 mice [10]. The improvements in insulin sensitivity, blood glucose, and HbA1c levels following chronic linagliptin treatment may be caused by reductions in liver triglyceride content and improvements in hepatic steatosis [10].

The effects on insulin sensitivity of combining two drugs with different mechanisms of action – chronic inhibition of renal glucose reabsorption and DPP-4 – have not been fully investigated. The aim of this study was to investigate the effects of 8 weeks' treatment with empagliflozin as monotherapy or in combination with linagliptin on whole body insulin sensitivity in *db/db* mice using euglycemic–hyperinsulinemic clamps. In addition, we investigated the effects of empagliflozin or linagliptin monotherapy or empagliflozin plus linagliptin combination treatment on glycemic control, liver fat content, and expression of key genes involved in metabolism and inflammation in the liver.

2. Materials and Methods

2.1. Experimental Animals and Study Design

Eight-week-old female *db/db* mice (n = 60) were purchased from Charles River (Boston, MA). After 1 week of acclimatization, mice were randomized into four different treatment groups: 10 mg/kg/day empagliflozin, 3 mg/kg/day linagliptin, a combination of 10 mg/kg/day empagliflozin plus 3 mg/kg/day linagliptin, and a vehicle control group which received Natrosol. The treatment period was 8 weeks. Empagliflozin and linagliptin (in 0.5% Natrosol) were administered orally once-daily between 08:00 and 09:00 using a cannula. Before randomization and during the treatment period, body weight, food intake, water uptake, fed plasma glucose, and HbA1c levels were measured once weekly. Experiments were performed in accordance with the rules for animal care of the local government authorities and were approved by the animal care and use committee of Leipzig University as well as by the animal care committee of the Bezirksregierung Leipzig, Germany (approval ID: TVV 27/08).

2.2. Oral Glucose Tolerance Tests

After 8 weeks of treatment, oral glucose tolerance tests (OGTTs) were performed after an overnight fast for 16 hours. Animals were orally loaded with 2 g/kg body weight glucose and tail vein blood was collected at 0 (baseline), 15, 30, 60, and 120 min following the glucose challenge. Blood glucose levels were measured using a glucometer (OneTouch® Ultra®; Lifescan, Milpitas, CA).

2.3. Euglycemic–Hyperinsulinemic Clamp Studies

To eliminate the empagliflozin-associated diuresis during the measurement, euglycemic–hyperinsulinemic clamp studies were performed 4 days after the last dose of treatment as described previously [10]. In brief, for catheter implantation, mice were anesthetized in the fed state 8 weeks after the start of treatment with an intraperitoneal injection of 240 mg/kg body weight Avertin® (2,2,2-tribromoethanol, 2-methyl-2-butanol; Sigma Aldrich, Hamburg, Germany). After loss of pedal reflex was confirmed, a catheter (Micro-Renathane® tubing, MRE025; Braintree Scientific, Braintree, MA) was inserted into the right internal jugular vein and advanced to the superior vena cava. The vein was then ligated distally. The catheter was filled with 100 μl of NaCl/heparin sulfate solution to prevent clotting. The end of the catheter was tunneled to the supra-scapular region. Mice were administered intraperitoneal injections of 1 ml saline containing 15 mg/g body weight of tramadol and placed on a heating pad to facilitate recovery.

Euglycemic–hyperinsulinemic clamps were performed on awake animals in the fed state 3 days after catheter implantation. After a 5-mCi bolus injection of D-[3-^{3}H]glucose (Amersham Biosciences, Little Chalfont, UK), the tracer was infused continuously (0.05 mCi/min) for the duration of the experiment. Baseline parameters were determined using a 50-μl aliquot of blood collected at the end of the 40-min run-in period. To minimize blood loss, red blood cells were collected by centrifugation, re-suspended in saline and re-infused. A bolus injection of insulin solution (40 mU/g; Actrapid 40U, Novo Nordisk, Copenhagen, Denmark) containing 0.1% BSA (Sigma-Aldrich) was followed by infusion at a fixed rate (4 mU/g/min). Blood glucose levels were determined every 10 min (B-Glucose Analyzer; HemoCue AB, Ängelholm, Sweden). Physiological blood glucose levels (between 120 and 150 mg/dl) were maintained by adjusting infusion of a 20% glucose solution (DeltaSelect, Rimbach, Germany). Approximately 60 min before steady state was achieved, a bolus of 2-deoxy-D-[1-^{14}C]glucose (10 mCi; Amersham Biosciences) was infused. Steady state was ascertained when glucose measurements were constant for ≥30 min at a fixed glucose infusion rate and was achieved within 120–150 min. During the clamp experiment, 5-μl blood samples were collected after infusion of 2-deoxy-D-[1-^{14}C]glucose at 0 and 5 min, and then