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Research Paper

Exploring glycosuria as a mechanism for weight and fat mass reduction. A pilot study with remogliflozin etabonate and sergliflozin etabonate in healthy obese subjects

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ABSTRACT

Inhibitors of sodium-dependent glucose co-transporter 2 (SGLT2) increase glucose excretion in the urine and improve blood glucose in Type 2 diabetes mellitus. Glycosuria provides an energy and osmotic drain that could alter body composition. We therefore conducted a pilot study comparing the effects on body composition of two SGLT2 inhibitors, remogliflozin etabonate (RE) 250 mg TID ($n = 9$) and sergliflozin etabonate (SE) (1000 mg TID) ($n = 9$), with placebo ($n = 12$) in obese non-diabetic subjects. Both drugs were well tolerated during 8 weeks of dosing, and the most common adverse event was headache. No urinary tract infections were observed, but there was one case of vaginal candidiasis in the RE group. As expected, RE and SE increased urine glucose excretion, with no change in the placebo group. All the subjects lost weight over 8 weeks, irrespective of treatment assignment. There was a reduction in TBW measured by D_2O dilution in the RE group that was significantly greater than placebo (1.4 kg, $p = 0.029$). This was corroborated by calculation of fat-free mass using a quantitative magnetic resonance technique. All but one subject had a measurable decrease in fat mass. There was significant between-subject variability of weight and fat loss, and no statistically significant differences were observed between groups. Despite a lack of a difference in weight and fat mass loss, the leptin/adiponectin ratio, a measure of insulin resistance, was significantly decreased in the RE group when compared to placebo and SE, suggesting that this SGTL-2 inhibitor may improve metabolic health independent of a change in fat mass.

Introduction

The incidence and prevalence of Type 2 diabetes (T2DM) are increasing as the result of a worldwide epidemic of obesity [1]. Medical management of patients with T2DM includes diet, exercise and weight reduction, together with oral anti-diabetic medications or insulin therapy, when appropriate [2]. Frequently, the treatment of T2DM now requires multiple agents acting via complementary mechanisms in an attempt to achieve tighter glycemic targets. Consequently, new agents with unique mechanisms of action and limited side effect profiles are needed when these targets cannot be reached [3].

Inhibitors of sodium-dependent glucose co-transporter 2 (SGLT2) reduce circulating glucose concentrations via a renal mechanism distinct from other current anti-diabetic agents [4]. SGLT2 is primarily expressed on the luminal side of the renal proximal tubule. It has high solute translocation capacity and low substrate affinity, and serves as the primary, but not exclusive, pathway for renal glucose reabsorption. Sergliflozin etabonate (SE) and remogliflozin etabonate (RE) are orally-active prodrugs of sergliflozin and remogliflozin, respectively. Sergliflozin is an SGLT2 inhibitor that increases urinary glucose excretion in a dose-dependent manner in rodents and dogs, and lowers plasma glucose levels following oral glucose challenge in diabetic rats [5]. Remogliflozin works similarly in mice and rats and exhibits antidiabetic efficacy in animal models and humans [6–8]. These two SGTL2 inhibitors have different effects on glucose excretion in humans. The maximal glycosuria observed with RE is greater than the maximal glycosuria achieved with SE [7,8].

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SGLT2 inhibitors improve plasma glucose concentrations and lower body weight in subjects with T2DM [8]. Administration of RE for 12 weeks to T2DM subjects resulted in a reduction of HbA1c of up to 1.07% versus placebo treatment, and a reduction in body weight of up to 3.51 kg (unpublished data). In another 12-week study conducted with T2DM subjects, dapagliflozin, a different SGLT2 inhibitor, improved HbA1c and produced weight changes of −2.5 to −3.4 kg compared to −1.2 kg for placebo [9]. No detailed body composition analyses were included in these studies to investigate the mechanism of the weight loss.

The renal glycosuria produced by SGLT2 inhibitors could alter body composition through loss of calories in the urine and by osmotic diuresis. In addition, initial weight changes during negative energy balance could be the result of diuresis caused by glycogen mobilization from the liver. If the sustained weight changes are the result of reduced adipose tissue stores caused by energy excretion as glucose, then this may explain, in part, the metabolic improvement seen with SGLT2 inhibitors.

The primary objective of this pilot study was to investigate the effect of RE and SE, administered for 8 weeks, on glucose excretion and body composition changes measured by quantitative magnetic resonance (QMR) [10], and by the 4-compartment (4C) body composition model [11]. In addition, we measured the changes in total body water (TBW) to determine the contribution of fluid loss caused by osmotic diuresis to the overall change in weight seen with these SGLT2 inhibitors.

Materials and methods

The study was conducted at the Addenbrookes Centre for Clinical Investigation (ACCI), Addenbrooke's Hospital, Cambridge, UK, in the GlaxoSmithKline Clinical Unit in Cambridge (CUC) and the Wellcome Trust Clinical Research Facility (WTCRF). The study was performed in accordance with Good Clinical Practice guidelines and the 1996 version of the Declaration of Helsinki and it was conducted between September 2006 and July 2007. The experimental protocols were approved by the protocol review panel at GlaxoSmithKline, the Cambridge Local Research Ethics Committee (06/Q0108/254) (EUDRACT 2006-003864-71), the Addenbrooke's Hospital R&D office, and by the WTCRF Scientific Advisory Board. All patients provided written informed consent prior to participation.

Thirty healthy subjects were recruited by direct advertisement. The sample size of this pilot study was based on feasibility. Enrollment criteria included body mass index (BMI) between 30 and 40 kg/m^2 and age between 18 and 55 years. Subjects with T2DM were excluded. The use of recreational drugs, alcohol, caffeine and strenuous exercise was forbidden. A qualified dietician advised subjects on a hypocaloric diet targeting a daily energy deficit of 2090 kJ [12] relative to the estimated daily caloric requirement of each subject, assuming a physical activity level of 1.3. After a 2 week run-in on diet alone, baseline 24 h urine glucose excretion, weight and fat mass were measured using QMR and 4C methods. Subjects were then assigned at random in a 3:3:2:2 ratio to SE 1000 mg three times daily [TID] ($n = 9$), RE 250 mg TID ($n = 9$), SE-placebo TID ($n = 6$), or RE-placebo TID ($n = 6$). Subjects were managed as outpatients and returned to the clinical unit every two weeks for eight weeks to receive counseling and to review safety endpoints. After eight weeks of dosing, they again underwent measurement of 24 h urine glucose excretion, weight and fat mass. Intermediate measurements of some pharmacodynamic endpoints were made at weeks 2, 4 and/or 6 and at a follow-up visit at week 12. Plasma sampling occurred at Week 6 to determine steady-state pharmacokinetics (PK) of RE, remogliflozin and GSK279782 (the main remogliflozin metabolite), and SE.

Fasting blood samples were collected at the beginning of the study and at clinic visits for leptin, adiponectin, IGF-1, VCAM-1.

Body composition measurements

Quantitative magnetic resonance

The characteristics of the QMR (Echo MRI-AH, Echo Medical Systems, Houston, TX, USA) have been described previously [10,13]. This methodology provides a rapid, non-invasive and highly precise measurement of human body fat using the nuclear magnetic resonance properties of protons to separate signals originating from fat and non-fat tissues. All QMR measurements were made in triplicate.

4-compartment model

Fat mass was also estimated using the following 4C equation [14]:

$$\text{Fat mass} = [2.747 \times \text{BV}] - [0.71 \times \text{TBW}] + [1.46 \times \text{BMC}] - [2.05 \times \text{BM}]$$

Where BV is body volume in liters and all other variables are in kilograms (TBW = total body water, BMC = bone mineral content, BM = body mass). BV was derived from a BOD POD™ (Life Measurement Inc, Concord, CA, USA) using estimates of % body fat and BM as follows [4]:

$$\text{BV} = (\%\,\text{fat} + 450) \times \text{BM}/495.$$

TBW was measured by D_2O dilution using a protocol designed in collaboration with the MRC Human Nutrition Research Group, Cambridge, UK, where the deuterium analysis was performed [15]. Subjects were fasted from 22:00 and at 06:00 the next morning they were awakened and asked to provide samples of saliva and voided urine for D_2O analysis. D_2O-enriched water (100 g of 7% by mass D_2O in H_2O) was then consumed, and further saliva samples were taken at 4, 5 and 6 h post dose. Up to the 6 h sample, the volume of all urine passed was recorded and assayed for D_2O content to correct for label lost from the body water pool.

Total body BMC was estimated from whole-body dual-energy x-ray absorptiometry (DXA) scans (GE Lunar Prodigy, software version 8.1 GE Lunar, Madison, WI USA).

Urinary glucose excretion

Urine was collected over 24-h intervals during clinic visits scheduled at Week 0, 2, 4, and 8, and urine samples were collected over 6-hour at Week 6 for measurement of drug concentrations. Aliquots taken from these urine collections were analyzed for urine glucose (molar units) and volume (milliliters). From these 2 measures, urine glucose excretion (mmol/24 h) and energy loss (kJ and kcal) were estimated. Urine energy loss was calculated from urine glucose excretion as follows:

Urine glucose in molar units was converted to grams by multiplying by 180.2. This value was then converted into energy in joules by multiplying by 15.76 and to kilocalories by dividing by 4.186. Urine glucose excretion on between the clinic visits was estimated using linear interpolation.

Hormone and peptide assays

The NIHR Cambridge Biomedical Research Centre, Core Biochemical Assay Laboratory analyzed the leptin and adiponectin using a two-site microtiter plate-based DELFIA assay [16], as well as IGF-1 (Siemens Healthcare Diagnostics) and VCAM-1 (Human VCAM-1 assay; R&D Systems Europe, Abingdon, UK).