Effects of agonists of glucagon like peptide - 1 receptors (GLP-1R) on arterial stiffness, endothelial glycocalyx and coronary flow reserve in patients with coronary artery disease and patients with diabetes mellitus

ClinicalTrials.gov Identifier: NCT03010683

Document date: December 22, 2016

Study Protocol

Objectives

Arterial stiffness is associated with increased risk for cardiovascular disease. Moreover, the integrity of endothelial glycocalyx plays a vital role in vascular permeability, inflammation and elasticity. Agonists of glucagon like peptide - 1 receptors (GLP-1R) used in the treatment of type 2 diabetes mellitus (T2DM). This category includes exenatide and liraglutide. These drugs lower glucose levels by inhibiting the secretion of glucagon, promoting the release of insulin in response to hyperglycemia, slowing gastric emptying, and augmenting satiety. Clinical studies have shown that GLP-1R agonists have beneficial effects on cardiovascular function in both diabetic patients and healthy subjects. The purpose of this study is to investigate in patients with T2DM without coronary artery disease (CAD), patients with T2DM and CAD and obese patients with abnormal oral glucose tolerance test (OGTT), changes in arterial stiffness,

endothelial glycocalyx thickness and coronary reserve flow (CFR) after treatment with metformin or agonist GLP-1R.

Design

The investigators will study three groups matched for age and sex: 30 patients with type 2 diabetes mellitus (T2DM) without coronary artery disease (CAD), 30 patients with T2DM and CAD and 30 obese patients (BMI >30 Kg/m^2) with abnormal oral glucose tolerance test (OGTT). It will be a randomized study with metformin or GLP-1R agonist treatment for 1 year. All subjects will receive for 1 year: (a) GLP-1R agonist or (b) metformin. At 0, 3, 6 and 12 months, where 0 is the starting point of treatment, blood samples will be collected.

Methods

At 0, 3, 6 and 12 months the investigators will measure:

a. Carotid-femoral pulse wave velocity (PWV, m/sec) using tonometry by Complior (SP ALAM) and augmentation index (AI, %) by the method of arteriography (Arteriograph, TensioMed)
b. Perfused boundary region (PBR, micrometers) of the sublingual arterial microvessels (ranged from 5-25 micrometers) using Sideview Darkfield imaging (Microscan, Glycocheck). Increased PBR is considered an accurate noninvasive index of reduced endothelial glucocalyx thickness.
c. Coronary flow reserve (CFR) in the left anterior descending artery after infusion of adenosine using Doppler echocardiography.
d. Determination of the following parameters in blood: glucose, insulin, free fatty acids, triglycerides, glycerol, C reactive protein (CRP), transforming

growth factor-b (TGF-b), Lipoprotein-Associated Phospholipase A2 (LP-LPA2), tumor necrosis factor-a (TNF-a), interleukins 6 and 10 (IL6 and IL10), propeptide of type I procollagen (PIP), propeptide of procollagen type III (PIIINP), matrix metallopeptidases 9 and 2 (MMP), macrophage-colony stimulating factor (MCSF), growth differentiation factor-15 (GDF-15), N-terminal pro b-type natriuretic peptide (NT-proBNP) and galectin-3.

Statistical analysis

All comparisons will be performed with the Statistical Package for Social Sciences 21.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Categorical data will be compared between patients by contingency tables. Continuous variables will be tested for normality using the Kolmogorov-Smirnov test. Normally distributed variables will be given as mean±standard deviation. Data with a non-gaussian distribution will be expressed as median (interquartile range) and will be analyzed after transformation into ranks. Differences in mean values for each of the measured variables will be compared by t-test or paired t-test for continuous variables. For non-normally distributed data Mann Whitney or Wilcoxon signed-rank test will be used. The investigators will use parametric (Pearson r) and non-parametric (Spearman rho) correlation coefficients to examine cross-sectional associations. ANOVA (general linear model, SPSS 22, SPSS Inc, Chicago, Ill) for repeated measurements will be applied a) for measurements of the examined markers at baseline, 3, 6 and 12 months after treatment used as a within-subject factor b) for the effects of treatment (liraglutide vs metformin), as a between-subject factors. The F and P

values of the interaction between time of measurement of the examined markers and type of treatment will be calculated. The F and P values of the comparison between treatments will be calculated. The Greenhouse-Geisser correction will be used when the sphericity assumption, as assessed by Mauchly's test, will not be met. Post hoc comparisons will be performed with Bonferroni correction.

Comparisons between baseline or post-treatment values of measured markers between the 2 treatment groups will be performed using factorial ANOVA. Post hoc comparisons will be performed with Bonferroni correction. Statistical significance will be considered as $p<0.05$. Baseline variables that will be statistically different ($p<0.05$) among the 2 study groups or will be of clinical significance (HbA1c, weight, BMI and waist circumference) will be included in multivariate models as covariates.