Study analysis plan (SAP)

A Randomized, Participant-blinded Five-arm Crossover Study with Blinded Outcome Assessment Investigating Glucagon's Effects on Heart Rate, Blood Pressure and Inotropy with and without Beta-blocker-induced Cardioinhibition

Short title: Glucagon+beta-blocker

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1. Administrative information

Study title

A Randomized, Participant-blinded Five-arm Crossover Study with Blinded Outcome Assessment Investigating Glucagon's Effects on Heart Rate, Blood Pressure and Inotropy with and without Beta-blocker-induced Cardioinhibition. EudraCT identifier: 2017–002613-58.

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2. Introduction: purpose and design

The purpose of this trial is to investigate the acute effects and mechanisms of intravenous glucagon (a recommended, but poorly explored antidote for beta-blocker poisonings (1)) on hemodynamics with and without hemodynamic suppression caused by an intravenous administration of the beta-blocker esmolol in healthy trial participants. Glucagon effects on catecholamine release, gastric emptying and adverse effect of glucagon are also explored. This trial is designed as a participant-blinded, five-arm, randomized cross-over trial with blinded outcome assessment, with a minimum one week washout period between trial days. Two glucagon infusion rates will be explored. Both esmolol and glucagon will be placebo-controlled (with matching saline placebo), thus, each participant will complete five days (esmolol+saline; esmolol+glucagon1 (50 µg/kg bolus injection(1)); saline+glucagon1; saline+saline; saline+glucagon2 (50 µg/kg bolus infusion over 30 minutes)) in random order. Esmolol is dosed in a dose sufficient to achieve >85 % beta-receptor occupancy (2–5). All hemodynamic endpoints will be recorded continuously by arterial cannulation of the radial artery in the wrist connected to a computer via a pressure transducer. A total of ten participants will be enrolled (see below). All participants

completing the five trial days will be included in the statistical analyses. For further information about trial contributors, trial design, arms, randomization and allocation and interventions; please see the clinicaltrials.gov-registration.

3. Study methods and statistical analyses

Randomization and blinding

Before enrolment, a randomization list allocating participants to a random intervention sequence (6) is generated by personnel not involved in the trial using an online tool (7). Upon inclusion by a blinded investigator, participants are numbered in sequence (1-10) – this number corresponds to a random intervention sequence on the list. This list is kept in a locked cabinet at the trial site. On trial days prior to interventions, personnel not involved in the trial will confer with the list and note interventions (A-E) on the specific day. The study is participant and outcome-assessor blinded: to avoid unblinding of participants, study drugs and placebos will be administered behind a curtain. Investigators and research nurses are not blinded to interventions on trial days. However, investigators and research nurses will not have access to the randomization list (unless code breach is necessary) and hence are unaware of interventions prior to the specific trial day.

Sample size

In previous pharmacodynamic studies with administration of 50 μg of glucagon, a change in heart rate of 10 heartbeats per minute (bpm) was observed. Power (1 - β) is set to 80%, where β (20%) is the risk of accepting a hypothesis that is false. The two-sided significance level (α) is set to 5%. Calculation of population size (N) was calculated using the formula below (8) and is based on the above-mentioned significance and power as well as an estimated minimum relevant difference of 10 bpm – our primary endpoint – and an estimated standard deviation of the difference (Σ) between two experimental days for the same volunteer of 7 bpm.

Power = pt (qt (0.025, N-1,0), n-1, - (μ1- μ2 / Σ) × √ (N)), μ1 - μ2 is the difference with / without glucagon (10 bpm). N is the number of subjects. The power calculation uses non-central t-function, pt(x,df,ncen) and its inverse qt. The above formula yields an 87% probability of finding a difference of 10 bpm when recruiting 8 subjects. We have chosen to recruit 10 to ensure a better opportunity for determining effects on endpoints.

Summary statistics

Screening (baseline) data will be summarized and presented by means of summary statistics (means, medians, standard deviations, interquartile ranges depending on distribution of data). Continuous data will be presented

with mean or median, standard deviation and/or standard error of the mean. Categorical data will be presented as counts and percentages (9).

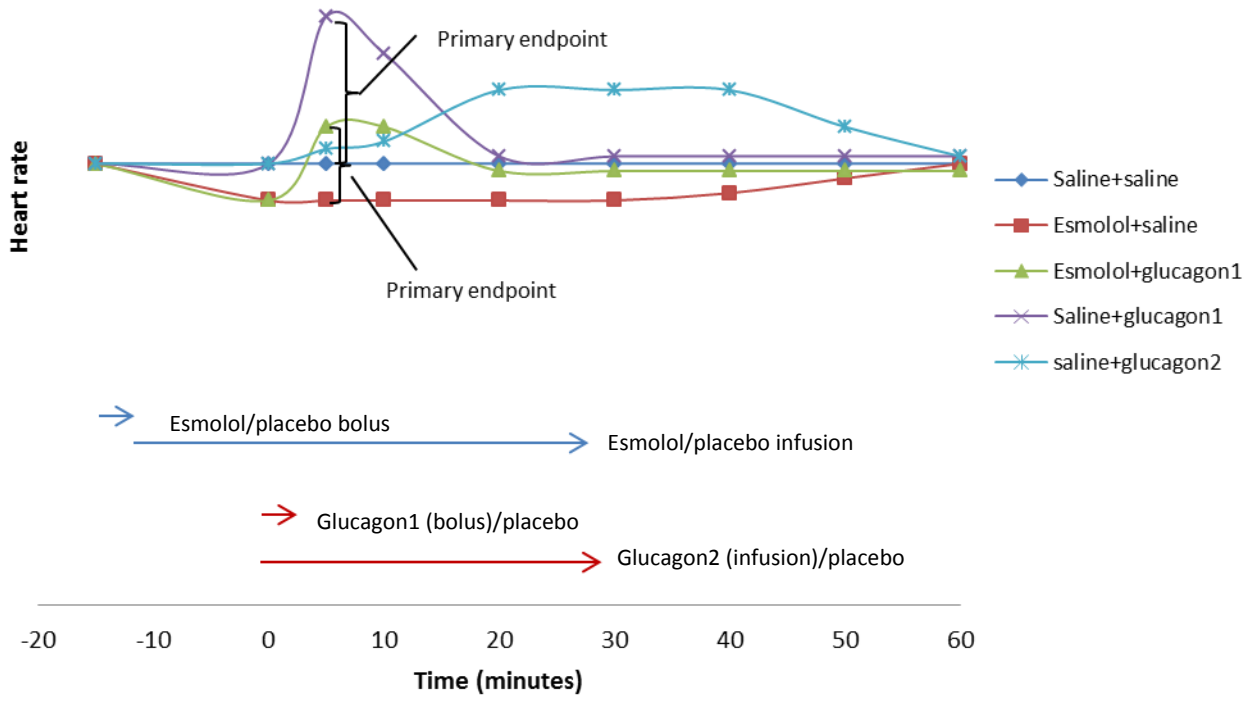
Endpoints and statistical analyses of endpoints

All endpoints will be analyzed blinded (blinded outcome assessment).

Analysis of the primary Endpoint (9)

The primary endpoint of this study is the difference in heart rate (change from baseline) on days with glucagon, compared to matching glucagon-placebo, 5 (4-6) minutes after glucagon/placebo (estimate and 95% confidence interval) (figure 1). To adjust for random fluctuation of the heart rate due to e.g. movement of the participant, we plan to use averages over a 2 minute time period. This difference can be analyzed using the paired t-test, repeat measures ANOVA or mixed models with fixed (intervention, period, timepoint) and random (participant ID) effects. If there is no carry-over, the paired t-test can be used for determination of the primary endpoint. Two-sided p-values <0.05 will be considered statistically significant.

Figure 1: Expected, approximate overview of effects of interventions and primary endpoints in the trial



Analyses of secondary/exploratory endpoints (9)

Secondary/exploratory endpoints

Secondary endpoints are changes from baseline in:

- heart rate,
- blood pressure,
- (relative) stroke volume,
- (relative) cardiac output,
- and corrected QT (QTc)-interval

compared between glucagon-days and days with corresponding placebo at the pre-specified timepoints (-20, -10, 0, glucagon +3, +5, +10, +15, +20, +30, +40, +50, +60 minutes – please also see the clinicaltrials.gov registration). QTcs will be calculated and compared between days using the formulae by Bazett or Fridericia depending on heart rate.

In addition, adverse events/discomfort due to glucagon compared to placebo will be evaluated at baseline and glucagon +6, +10, +30, +60 minutes (10,11). Further, participants will be administered 1.5 g paracetamol (acetaminophen) prior to glucagon/placebo. Blood will be drawn at baseline and glucagon +10, +20, +30, +40, +50, +60 minutes for measurement of paracetamol pharmacokinetics (maximum concentration, time of maximum concentration, area under the curve) using this as marker of gastric emptying time (12).

This trial involves further exploratory endpoints: glucagon plasma concentrations and blood glucose. For pre-specified timepoints of these secondary endpoint measurements and comparison between glucagon- and placebo-days, please see the clinicaltrials.gov-registration. Plasma norepinephrine is measured at T-20, T0, and glucagon +5, +30 and +60 minutes.

Statistical analysis of secondary/exploratory endpoints

Continuous secondary/exploratory endpoint data will be presented using scatter plots of the mean or median with locally weighted scatter plot smoothing (LOWES) curves. Changes from baseline with 95% CIs will be compared using appropriate statistical methods, e.g. mixed model for repeated measures or repeated measures ANOVA in addition to the descriptive statistics and scatter plots (due to the paired design). Two-minute averages will be used for comparison at the timepoints, in order to handle random fluctuation of the endpoints measurements (e.g. timepoint 30 = averages form 29 to 31 minutes). Percentage change from baseline will be calculated for relative stroke volume and cardiac output derived from the pulse contour curve using the Liljestrand-Zander equation (SV = k × (pulse pressure / (systolic blood pressure + diastolic blood pressure) (13),

and presented as percentage point differences. Ordinal data will be analyzed and compared between days using Wilcoxon signed rank-test (due to the paired design (9)). Adjustment of multiple comparisons will be performed (method used will be reported). Two-sided p-values <0.05 will be considered statistically significant.

Statistical software
Statistical analyses will be performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA), R (version 3.2.3) (14) and GraphPad Prism 7.02 (GraphPad Software Inc., La Jolla, CA, USA). Graphs will be made using GraphPad Prism 7.02.

Harms
All adverse events will be summarized and presented in a table along with investigator's assessment of causality, and in accordance to CONSORT extension for harms (15), in addition to the secondary endpoint on nausea described above.

Missing values
Few missing (completely at random) values are expected. These will be handled by mean substitution or alternatively multiple imputations. The method used will be reported.

Interim analyses
Due to the exploratory nature of this study, an interim analysis for evaluation of study methods and tolerability – using the above stated statistical methods for analysis of endpoints – is planned after the two first enrolled participants each have completed all five trial days. The trial is continued without change if the treatments are well-tolerated. Trial protocol changes will be reported as required to the regulatory bodies (the Danish Medical Agency and the local scientific ethics committee) and documented on clinicaltrials.org and in the SAP.

4. Trial population

Eligibility criteria
This trial includes healthy volunteering men, 18 to 40 years of age, determined healthy by medical history, physical examination including laboratory screening tests. Screening data will be presented as described above (summary data).

Inclusion criteria:
• Healthy male determined by investigator, based upon physical examination, medical history, ECG, vital signs and laboratory results

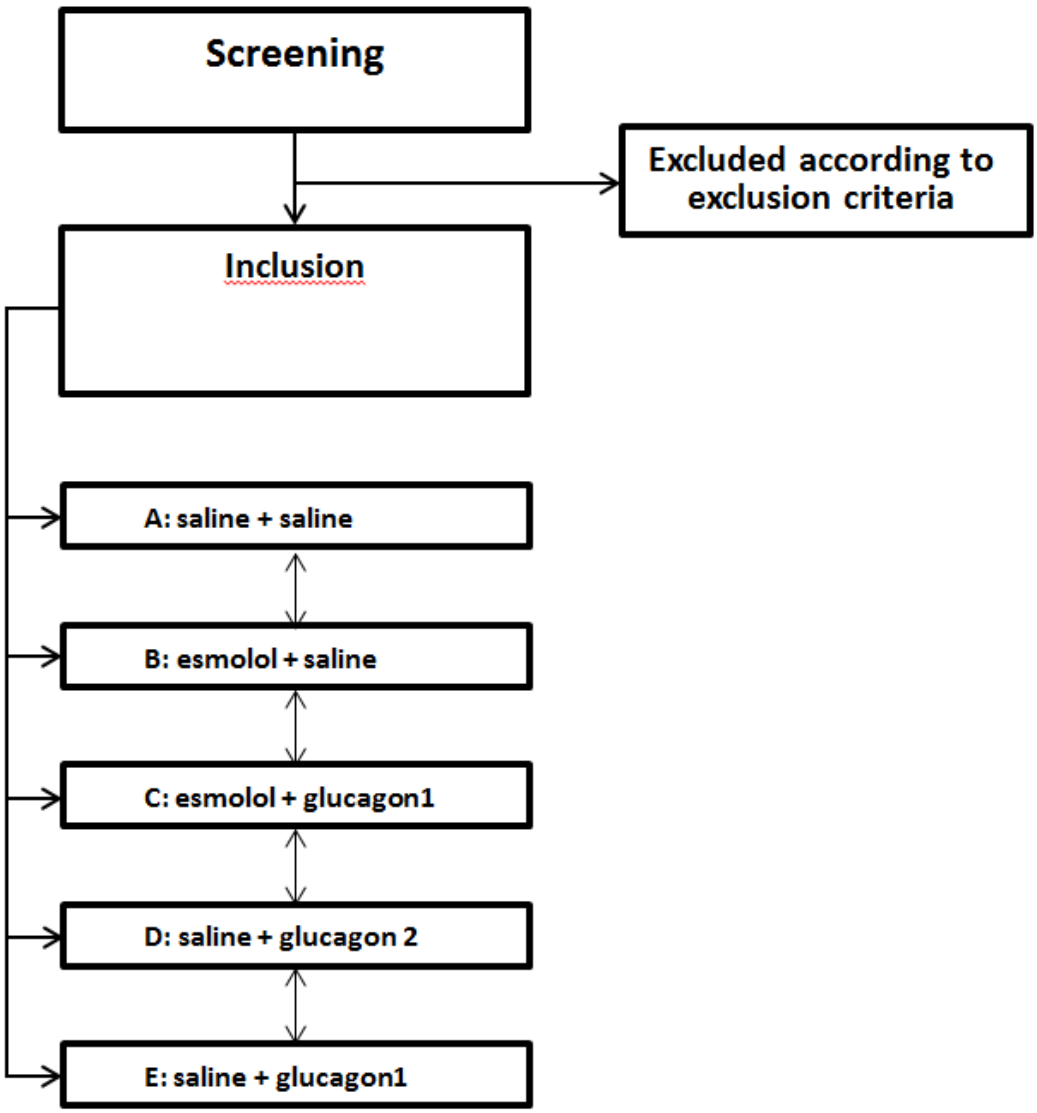
• Body mass index (BMI) ≥ 18.5 and ≤ 29.9 kg/m2 and body weight between 50 and 100 kg, inclusive, at screening visit.

Exclusion Criteria:
• Abnormal blood levels of sodium, potassium, creatinine, alanine transaminase (ALT), alkaline phosphatase, albumin, bilirubin, haemoglobin, HbA1c, cholesterol fractions.
• Bradycardia (<45 beats per minute)
• Hypotension (systolic blood pressure < 100 mmHg)
• Second or third degree atrioventricular conduction delay
• Sick sinus syndrome
• Any heart disease or hypertension
• Pheochromocytoma
• Allergy to any active or inactive ingredient contained in investigatory medicines or tools.
• Raynaud's syndrome
• Prinzmetal's angina
• Diabetes
• Pulmonary disease
• Pheochromocytoma
• Any contraindication against investigatory medicines or tools.

Dropout/withdrawal
A participant is withdrawn if he experiences unacceptable side effects, starts any pharmacological treatment (excluding the occasional pain or allergy medication) or declines to participate further. If a participant is withdrawn for any reason, a new participant will be screened and included.

Presentation of baseline characteristics and flow chart
All information regarding participant flow, e.g. the number of participants screened and included or excluded (with reason for exclusion) will be presented in a CONSORT flow diagram (please see figure 2).

Figure 2. Trial flow chart



5. Conflicts of interests

No contributors involved in trial planning, conduction and data analysis have any conflicts of interests to declare.

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