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Inhibiting C-Reactive Protein Synthesis by Cardiac Glycosides in Humans

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Abstract: The role of C-reactive protein (CRP) in cardiovascular disease has been controversially discussed for almost two decades. Specific CRP inhibition, followed by use of CRP inhibitors in controlled clinical trials may be the only way to prove or disprove a causative role for CRP in cardiovascular disease. Endogenous and plant-derived inhibitors of the Na(+)/K(+)-ATPase, *i.e.* the cardiac glycosides ouabain, digoxin and digitoxin, potently inhibit CRP synthesis in human hepatoma cells and primary human hepatocytes *in vitro.*

In the herein described single-center **C**-reactive protein-**D**igoxin **O**bservational Study (C-DOS), 60 patients with decompensated heart failure, NYHA III-IV and severely reduced Left Ventricular Ejection Fraction (LVEF<40%), and elevated CRP plasma levels will be treated by either digoxin+conventional heart failure therapy (30 patients) or by conventional heart failure therapy alone (30 patients). Plasma CRP levels in both groups will be assessed for 21 days. Plasma CRP levels (day21-day0) will be compared by regression analysis in order to find out whether digoxin significantly lowers CRP plasma levels in humans.

The trial will not answer the question whether CRP is causative in cardiovascular disease but, by following a step by step approach, investigates for the first time whether cardiac glycosides lower CRP plasma levels in humans. The study hereby serves as a pilot study for subsequent phase III trials. Importantly, it is the first trial ever that systematically uses a direct CRP synthesis inhibitor *in vivo* in humans.

Keywords: C-reactive protein, C-reactive protein synthesis, cardiovascular disease, cardiac failure, clinical study, drug development.

1. INTRODUCTION

In this article we summarize background, design aspects, statistical analysis and ethical considerations of our C-reactive protein-Digoxin Observational Study (CDOS), that has been discussed in detail on Drug Discovery & Therapy World Congress 2015 (DDTWC 2015), Boston, MA, USA http://www.ddtwc.com/.

2. BACKGROUND

Elevated plasma levels of C-reactive protein (CRP), the prototype acute-phase protein (APP), are predictive for future cardiovascular events [1, 2]. The role of CRP as a risk factor contributing to the pathogenesis of atherosclerosis is controversial [3, 4]. Whereas initial experimental studies suggested a pathogenic role for CRP in atherogenesis [5], more recent genetic data from Mendelian randomization trials failed to provide evidence for a causative role of CRP in cardiovascular disease [6, 7]. Mendelian randomization trials, however, do have limitations [8, 9]. Also, experimental results from laboratories all over the world have been contradictory, partly because of species differences in CRP biology [4] (which may limit the value of animal models in this particular area of research) and partly because data were not accurately evaluated [10, 11]. Compelling evidence suggests that CRP activates the complement system and binds to and activates Fcγ receptors in macrophages in atherosclerosis and thereby sustains a chronic inflammatory

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process in the arterial wall [4, 14].

By analogy with the history of low density lipoprotein, HMG-CoA-enzyme inhibitors and cardiovascular disease [12], specific CRP inhibition, followed by use of CRP inhibitors in controlled clinical trials may be the only way to prove or disprove a causative role for CRP in cardiovascular disease [3].

As a result of a high throughput screening assay (HTS) to investigate the effect on CRP transcription of well-defined classes of pharmacological agents, we have shown in 2010 that endogenous and plant derived inhibitors of the Na(+)/K(+)-ATPase, *i.e.* the cardiac glycosides ouabain, digoxin and digitoxin, inhibit IL-1beta- and IL-6-induced acute phase protein expression in human hepatoma cells and primary human hepatocytes at nanomolar concentrations [13]. Whether this *in vitro* finding holds true *in vivo* in humans and is also detected at CRP plasma level needs to be tested.

3. THE NEED FOR A TRIAL

Specific CRP inhibition, followed by use of CRP inhibitors in controlled clinical trials may be the only way to prove or disprove a causative role for CRP in cardiovascular disease [3, 4]. An effect of cardiac glycosides on CRP plasma levels in humans has never been reported. Notably, in a recent seminal meta-analysis digoxin has been demonstrated to be associated with a neutral effect on mortality in randomised trials and a lower rate of admissions to hospital across all study types [15]

Patients with decompensated heart failure, NYHA III-IV and severely reduced Left Ventricular Ejection Fraction (LVEF<40%) will be assessed for eligibility. In these patients, the plasma CRP is elevated [16]. Such study is ethically justified, because cardiac glycosides have been used in cardiac insufficiency for 230 years [17] and, according to the heart failure guidelines, still provide an additive treatment option in NYHA classes III and IV [18]. Thus, treatment with cardiac glycosides in NYHA III and IV patients is in line with heart failure guidelines and ethically justified.

The proposed trial will not answer the question whether CRP is causative in cardiovascular disease but, following a step by step approach, investigates for the first time whether cardiac glycosides lower CRP plasma levels in humans. The study serves as a pilot study for subsequent phase III trials.

4. JUSTIFICIATION OF DESIGN ASPECTS

The study design has been extensively discussed with the Ethical Review Committee of Ulm University. Whereas the study was initially designed as a blinded, randomized clinical trial with two groups (conventional heart failure therapy plus digoxin vs. conventional heart failure therapy alone), the Ethical Review Committee suggested to change the design in order to avoid that final medication (digoxin or not) depends on the patient`s participation in the study. Following the Ethical Review Committee`s advice we have designed an observational study/prospective cohort study, which was finally accepted by the Ethical Review Committee.

4.1. Controls/Comparators

CRP plasma levels of 30 patients with decompensated heart failure, NYHA III-IV, severely reduced Left Ventricular Ejection Fraction (LVEF<40%) and conventional heart failure therapy plus digoxin will be compared to CRP plasma levels of 30 patients with decompensated heart failure, NYHA III-IV, severely reduced Left Ventricular Ejection Fraction (LVEF<40%) and conventional heart failure therapy alone.

4.2. Inclusion and Exclusion Criteria

Inclusion: Age>18 yrs; NYHA III and NYHA IV; acute cardiac failure (acute worsening of dyspnoe, radiological signs of cardiac congestion); left ventricular ejection fraction<40% in echocardiography (2 observers, Teichholz and Simpson method)

Exclusion: Significant concomitant disease (acute coronary syndrome, infection, antibiotic therapy, acute renal failure, cancer, autoimmune disease); CRP>5mg/dl, leukocyte count>12000/μL, body temperature >38 °C; AV-block I-III (for Digoxin patients).

Explanation for exclusion criteria: An exclusion of CRP>5mg/dl, leukocyte count>12000/μL, body temperature >38°C avoids confounding influence of infection. Due to the expected significant proportion of patients that need to be excluded, ~800 patients will be assessed for eligibility. Due to the expected high drop-out rate, ~80 patients will be assigned to the trial, and only 60 patients will finally be included in the trial.

4.3. Outcome Measures

CRP (and digoxin) plasma levels at days: 0, 2, 4, 6, 8 and 21. Thus, Primary efficacy endpoint is CRP plasma level during follow-up (day21 – day0). Key secondary endpoint(s): NTproBNP plasma levels (day21 – day0).

Explanation for follow up period: CRP plasma half-life is about 19h in humans. Therapeutic blood concentration of digoxin is reached after 3 days. We assumed that, after 21 days, a potential effect of digoxin on CRP synthesis should be visible.

4.4. Methods Against Bias

Echocardiography (EF) will be analysed by two investigators *via* Teichholz and Simpson method. Multiple regression analysis will be used to adjust for potential confounding due to gender, age, cardiac rhythm or center-specific effects. CRP plasma levels will be assessed by the independent clinical laboratory (Clinic Association Kempten-Oberallgäu, Kempten, Germany) via routine CRP measurement. Per protocol analysis of n=60 (30 digoxin vs. 30 control) patients will be performed as well as intention-to-treat analysis of approximately 80 patients assigned to the trial. Blinding is not possible because intervention and control depends on clinical needs. Digoxin plasma levels need to be monitored for safety reasons because of the drug`s small therapeutic window.

4.5. Proposed Sample Size/Power Calculations

Power calculation was discussed with the Institute of Epidemiology and Medical Biometry of Ulm University. There has never been any study investigating the effect of cardiac glycosides on CRP plasma levels before and thus, biometrical classification of the study is “pilot study for subsequent phase III trials”. The sample size of 60 patients in total was evaluated as being adequate to apply the aforementioned multiple regression analysis with 4 confounders. The expected drop-out rate is high due to, for example, acquirement of lung infection following cardiac decompensation, other infectious diseases or bradycardia due to digoxin treatment. There was no data base to conduct a formal sample size calculation due to the lack of retrospective trials in the field.

4.6. Feasibility of Treatment

Decompensated heart failure is one of the most common diagnoses on admission in cardiovascular units [18]. All the admitted patients will be screened for inclusion and exclusion criteria. Feasibility and safety of recruitment is definitely given, because cardiac glycosides have been used in cardiac insufficiency for 230 years [17] and, according to the heart failure guidelines, still provide an additive treatment option in NYHA classes III and IV [18]. Expected finalization of patient recruitment is December 2015 based on current recruitment rate.

5. STATISTICAL ANALYSIS

After final data acquisition, all variables will be descriptively analyzed. To assess the efficacy of the investigated treatment scheme, multiple regression analysis will be performed. The dependent variable is the difference of CRP plasma level at day 21 and baseline, the independent variables are the group status (digoxin *vs.* placebo) and the four confounding variables gender, age, cardiac rhythm, i.e. sinus rhythm vs. atrial fibrillation, and study center. The level of significance is set to 5% (two-sided). The analyses of all secondary endpoints will be conducted in an explorative manner. Analyses concerning safety issues will be done by evaluating the adverse events frequencies in both groups. The expected drop-out rate (50%) is high, due to, for example, potential acquirement of infection during follow-up. Per protocol analysis of n=60 (30 digoxin *vs.* 30 control) patients will be performed as well as intention-to-treat analysis of approximately 80 patients assigned to the trial.

CONCLUSION

This is the first trial ever that systematically uses a direct CRP synthesis inhibitor *in vivo* in humans.

CONFLICT OF INTEREST

The study is funded by the Clinic Association Kempten-Oberallgäu. There is no conflict of interest for any of the authors.

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