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Validation of a pharmacological model for mitochondrial dysfunction in healthy subjects using simvastatin: A randomized placebo-controlled proof-of-pharmacology study

Marcus P.J. van Diemen[a,*], Cécile L. Berends[a], Naila Akram[a], Joep Wezel[b], Wouter M. Teeuwisse[b], Bert G. Mik[c], Hermien E. Kan[b], Andrew Webb[b], Jan Willem M. Beenakker[b], Geert Jan Groeneveld[a,1]

[a] Centre for Human Drug Research, Zernikedreef 8, 2333 CL Leiden, The Netherlands
[b] C.J. Gorter Center for High-field MRI, Albinusdreef 2, 2333 ZA Leiden, The Netherlands
[c] Erasmus Medical Center, Department of Anesthesiology, 's-Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands

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ABSTRACT

Proof-of-pharmacology models to study compounds in healthy subjects offer multiple advantages. Simvastatin is known to induce mitochondrial dysfunction at least partly by depletion of co-enzyme Q10. The goal of this study was to evaluate a model of simvastatin-induced mitochondrial dysfunction in healthy subjects and to determine whether mitochondrial dysfunction could be pharmacologically reversed by treatment with co-enzyme Q10 (ubiquinol).

Subjects received simvastatin 40 mg/day for 8 weeks. After 4 weeks, subjects were randomized to receive ubiquinol 300 mg/day or placebo in a double-blinded fashion. Mitochondrial function was assessed by measuring the phosphocreatine recovery time (τ-PCr) using phosphorous Magnetic Resonance Spectroscopy (^{31}P-MRS) after in-magnet exercise.

After 4 weeks of simvastatin treatment, τ-PCr prolonged with 15.2% compared to baseline, (95%CI, 2.5–29.4%; P = 0.018, Fig. 3). After 8 weeks, τ-PCr further prolonged to 37.27 s in the placebo group (prolongation of 18.5% compared to baseline, still significantly prolonged, 95%CI, 1.1–38.9%; P = 0.037), but shortened to 33.81 s in the ubiquinol group (prolongation of 9.1% compared to baseline, no longer significantly prolonged, 95%CI, −7.9 to 29.2%; P = 0.31). At 8 weeks, there was no significant difference between groups (difference of 8.2%, 95%CI, −14.5 to 37.0%; P = 0.51).

Simvastatin induces subclinical mitochondrial dysfunction in healthy subjects, which can be partly reversed by treatment with ubiquinol. This model of pharmacologically induced and reversed mitochondrial dysfunction can be used to study the effects of compounds that enhance mitochondrial function in healthy subjects.

1. Introduction

Evidence is growing that dysfunctional mitochondria play a central role in many age-related diseases, such as neurodegenerative diseases, sarcopenia and type 2 diabetes (Saft et al., 2005; Victor et al., 2011; Konopka and Sreekumaran Nair, 2013; Yan et al., 2013). The burden of age-related diseases on elderly and society are significant: in 2000, estimated healthcare costs attributable to sarcopenia in the United States alone were $18.5 billion (Janssen et al., 2004). Finding new and innovative drug targets in this population is much needed. Mitochondrial dysfunction (MD) is therefore becoming an increasingly important drug target for development by the pharmaceutical industry (Andreux et al., 2013).

Proof-of-Pharmacology (PoP) studies are designed to identify the viability of candidate molecules for full clinical development in an early phase, by detecting pharmacology on a pathophysiologically relevant mechanism (Cohen et al., 2015). To keep inter-subject variability at a minimum and drug development costs lower, a PoP study is ideally conducted in healthy subjects. A challenge model, pharmacological or non-pharmacological, is typically used, such as scopolamine to induce lower than normal cognitive function, or tryptophan depletion to induce a depressed mood (Baakman et al. 2015; Gijsman et al., 2002). No

* Corresponding author.
E-mail address: mvdiemen@chdr.nl (M.P.J. van Diemen).
[1] Principal Investigator.

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challenge model yet exists to study mitochondrial dysfunction. Here, we describe a model in healthy subjects, using simvastatin to induce MD and subsequently ubiquinol, the reduced form of co-enzyme Q10 (CoQ10), to reverse it. The induction of MD by statins is based on work from Wu et al., who showed MD in statin users after restarting their therapy, reportedly by inhibition of the biosynthesis of CoQ10 which is the main electron carrier in the mitochondrial electron transport chain (ECT) (Diebold et al., 1994; Littarru and Langsjoen, 2007; Bouitbir et al., 2011; Wu et al., 2011). Reversibility of the induced effect, to be able to assess the pharmacological effect of the candidate drug or food compound can be shown, is an important additional step for a PoP model (Wu et al., 2011). Statins have been reported to cause MD by down-stream inhibition of the CoQ10 biosynthesis (Diebold et al., 1994; Littarru and Langsjoen, 2007; Bouitbir et al., 2011). CoQ10 functions as electron carrier in the mitochondrial electron transport chain (ECT). Primary and secondary deficiencies of CoQ10 result in clinical disease, typically affecting muscular and neurological systems, which highly dependent on mitochondria for energy (Quinzii and Hirano, 2011)

We used 31-phosphorus Magnetic Resonance Spectroscopy (^{31}P-MRS) as gold standard to determine the phosphocreatine (PCr) recovery time (τ-PCr), which has been validated by in vitro respirometry (Bendahan et al., 2006; Lanza et al., 2011). We also determined mitochondrial function using several less burdensome and cheaper alternatives. Oxygen consumption has been proposed to reflect mitochondrial function (Kemp et al., 2001; Ryan et al., 2013). We determined the oxygen consumption rate (mVO_2) in muscle tissue, using Near Infrared Spectroscopy (NIRS), and mitochondrial oxygen tension ($mitoPO_2$) in the skin, using Protoporphyrin-9 Triplet State Lifetime Technique (PpIX-TSLT). We hypothesized to induce subclinical MD in healthy subjects and to show the pharmacological effect of ubiquinol in reversing the induced MD.

2. Materials and methods

The study was conducted as a single center, randomized, double-blind, parallel, placebo-controlled trial. The subject number was estimated based on published study by Wu et al. in 10 statin users, in which 4 weeks of treatment with a statin (simvastatin 20 or 40 mg/day, atorvastatin 5 or 10 mg/day or rosuvastatin 5 mg/day) led to MD measured by ^{31}P-MRS (Wu et al., 2011). In this study, the mean τ-PCr increased from 28.1 to 55.4 s with an SD of 23.4. The assumption underlying our hypothesis was that ubiquinol suppletion for a period of 4 weeks would completely restore mitochondrial function and would therefore lead to a complete return to baseline τ-PCr. In order to demonstrate a difference in mean τ-PCr between ubiquinol and placebo of 27.5 s, at least 12 subjects per treatment arm were needed assuming that the common standard deviation is 23, using a two group *t*-test with a 0.05 two-sided significance level. Because of potential drop-outs, a sample size per treatment arm of $n = 14$ was chosen.

Thirty subjects were included (14 females and 14 males, Fig. 1), with two subjects dropping out within two weeks after study start. Subjects were medically screened up to 28 days prior to study enrolment for eligibility. Inclusion criteria included; aged between 40 and 70 years and BMI 18–32 kg/m^2. Exclusion criteria included a clinically relevant disease; clinically significant abnormalities on routine chemistry and haematology laboratory; plasma creatine kinase (CK) levels > 145 U/L (for females) or > 170 U/L (for males); history of myopathy; diabetes mellitus and/or lower extremity peripheral vascular disease; recent (within 14 days) use of medications with known mitochondrial toxicity (i.e. metformin, statins, paracetamol and Non-Steroidal Anti-Inflammatory Drugs) and vitamin supplements; any contraindication to have a MRI scan; pregnancy in females; a history (within 3 months of screening) of alcohol consumption exceeding 2 units per day on average; a sedentary lifestyle; smoking within 12 h of the study visits; alcohol consumption within 24 h of the study visits; and excessive physical activity within 48 h of the study visits. The study was approved by the independent ethics committee Stichting Bebo (Assen, the Netherlands) according to the principles of the Helsinky Declaration under number NL48758.058.14, and informed consent was obtained from all subjects.

At study enrolment, the subjects were fully randomized by an independent and unblinded statistician, using a random seed in SAS for Windows V9.4 (SAS Institute, Inc., Cary, NC, USA), within two blocks of 14 subjects (ubiquinol or placebo), which were stratified for sex. Jars containing study medication and matching placebos were prepared and labeled by an unblinded pharmacy. The blinded study-physician enrolled the subjects by awarding subject numbers, which were linked to a randomization code. All subjects were treated with film-coated simvastatin 40 mg tablets (Teva Pharmaceutical Industries Ltd, Petah Tikva, Israel) daily for 8 weeks. The dose was chosen to keep adverse effects, most notably statin-associated myopathy, at a minimum based on a large clinical trial comparing simvastatin 20–80 mg (Armitage et al., 2010). After 4 weeks of simvastatin treatment, ubiquinol 300 mg capsules (Kaneka QH, Kaneka Corporation, Japan) or matching placebo were administrated daily in parallel for the remaining 4 weeks. Ubiquinol 300 mg was chosen, due to the superior bioavailability of ubiquinol and proven safety for a dose up to 300 mg (Langsjoen and Langsjoen, 2014). All dosings were orally administered by the subjects at home around dinner time with sufficient still water. Times and dates of administration were noted by the subjects in a medication diary. Compliance with the drug regimen was checked by pill count during each study visit. Throughout the study, subjects with complaints of severe myopathy were excluded.

Subjects were admitted to the Clinical Research Unit of the Centre for Human Drug Research (CHDR, Leiden, the Netherlands) at day 0 (baseline visit before simvastatin treatment), day 14, day 28 (baseline visit before and ubiquinol/placebo treatment) and day 56 (end of treatment period). Measurements (^{31}P-MRS, NIRS, PpIX-TSLT and Jamar dynamometry) were performed during all 4 visits. The ^{31}P-MRS measurements were performed at the Gorter Center for high-field MRI (Leiden University Medical Center, Leiden, the Netherlands). Subjects were contacted by telephone no longer than 10 days after the last visit. Adverse events and concomitant medications were continuously registered throughout the entire study period. Plasma creatine kinase (CK) was measured at baseline, day 14, day 28 and day 56 to monitor subclinical signs of statin-induced myopathy.

2.1. Phosphorus Magnetic Resonance Spectroscopy (^{31}P-MRS)

^{31}P-MRS was performed on a 7-tesla MRI scanner (Phillips, Best, The Netherlands) on the right posterior calf, using a custom-built 8 × 6 cm ^{31}P surface coil. A MRI-compatible pedal allowed the subjects to perform isometric plantar flexion exercise while supine. The right foot was strapped firmly to the pedal using non-elastic Velcro straps proximal to the base of the fifth digit with the right knee supported. Additional straps across the mid-thigh and mid–lower leg assured to isolate usage of the posterior calf muscles. Subjects were instructed to near-maximally contract the calf muscles, decreasing the PCr levels to around 50% of baseline, which could be monitored real-time by the investigator. Exercise took 3 min with rest intervals between plantar flexions (2.5 s contraction, 1.5 s rest), in order to keep changes to the blood flow to a minimum. The scanning protocol consisted of localizer sequences and the acquisition of a field map for shimming purposes using a custom-built outer partial volume coil, tuned to the proton frequency. Thereafter, ^{31}P-MRS data were acquired before, during and after exercise with a time resolution of 1 s.

Peak integrals of the inorganic phosphate (Pi), PCr and ATP signals were obtained using the jMRUI software package (version 5.0, jMRUI Consortium). The frequency difference between PCr and Pi was used to calculate tissue pH. Recovery curves were fitted to a mono-exponential function to determine the τ-PCr using a custom made MatLab script (version 2012b). Outlying data, deviating more than 5% from the