Bezafibrate as treatment in males for Barth syndrome: CARDIOMAN, a double-blind, placebo-controlled crossover RCT

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Scientific summary

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Scientific summary

Introduction

Barth syndrome is a very rare, life-threatening, X-linked recessive genetic disease almost exclusively affecting young males, caused by abnormal lipids in mitochondria. The tafazzin gene (*TAZ*) encodes tafazzin, a phospholipid acyltransferase. Tafazzin transfers unsaturated fatty acids with acyl chains from phospholipids to monolysocardiolipin (MLCL) and regulates remodelling and maturation of cardiolipin, a phospholipid located exclusively in the inner mitochondrial membrane. Mature tetralinoleoyl-cardiolipin (L4-CL) is essential in the mitochondrial membrane for maintaining mitochondrial membrane potential, structural integrity of electron transport chain complexes and mitochondrial cristae architecture.

Cardiolipin is a major constituent of inner mitochondrial membranes, and the major effects of the aberrant gene are on muscular tissues. Cardiac and skeletal manifestations of Barth syndrome are thought to result from impaired formation of respiratory chain super-complexes. *TAZ* mutations decrease L4-CL and increase intermediate species of MLCL, resulting in a marked increase in the MLCL : L4-CL ratio, which provides a highly sensitive and specific biochemical test for Barth syndrome. Barth syndrome is characterised by many gene mutations and profound phenotypic variability between affected patients within families and those in different families.

The disease carries many risks and problems for its sufferers, as well as major healthcare costs. Almost one-third (30%) of living UK patients have undergone cardiac transplantation. Several patients who have died due to cardiac rejection or post-transplant lymphoproliferative disease had previously undergone cardiac transplantation. Other patients who have died deteriorated too fast to undergo transplantation or died of complications while awaiting transplantation.

Neutropenia is another life-threatening issue. Two-thirds of UK patients are treated with granulocyte colony-stimulating factor by subcutaneous injection. Managing neutropenia in this disease is challenging, since patients have highly variable neutrophil counts, which prevents administration of a consistent daily dose and requires repeated blood counts and physician oversight.

Exercise intolerance, lethargy and fatigue are universal, interfering with daily life, schoolwork and play and often necessitating use of a wheelchair. Patients cannot perform strenuous or demanding jobs. Many have major feeding problems from infancy through to adult life; for example, requiring feeding via gastrostomies.

There can be rapid deterioration during periods of stable health despite expert medical care. Ventricular arrhythmia (tachycardia or fibrillation) affects 10% of adolescents and can cause sudden cardiac death at any time.

There are no specific treatments for Barth syndrome. There is no evidence that standard medications for cardiomyopathy ameliorate long-term poor outcomes. Treatment is multidisciplinary and currently limited to treating intercurrent bacterial infections, bone marrow support, physiotherapy, nutritional support, management of heart failure and arrhythmia, and consideration for heart transplantation.

Experiments using lymphoblasts from patients with Barth syndrome show that either bezafibrate or resveratrol can partially normalise the deranged cardiolipin ratio. This may be important, since some patients with Barth syndrome who lack neutropenia and tend to have good exercise tolerance have cardiolipin ratios intermediate between those of typical patients and normal individuals. Bezafibrate also

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improved left ventricular function and showed other benefits at supraphysiological doses in a *TAZ* knockdown mouse model.

This trial was motivated by the need for disease-specific therapy to prevent morbidity, mortality, psychological distress, and disruption of quality of life (QoL) in affected patients and their families. An effective therapy also has the potential to generate savings for the NHS.

Bezafibrate is a lipid-lowering drug used in adults and children, with a good safety record in long-term use. These characteristics made it a candidate for investigation. Bezafibrate has been reported to significantly ameliorate a mitochondrial myopathy in adults and shown promising results in several animal/human cellular models of mitochondrial disease.

Several of the laboratory assays and clinical investigations proposed in this study were also considered potentially important beyond the scope of this specific study, to assess associations between genotype and phenotype and for future evaluations of therapies in Barth syndrome.

Objectives

- Estimate the effect on clinical, biochemical and QoL outcome measures of bezafibrate compared to placebo.
- Investigate whether clinical improvements parallel in vitro changes in cardiolipin ratio and mitochondrial morphology in each participant's cells when cultured with bezafibrate.
- Investigate whether clinical improvements and culture findings with bezafibrate parallel in vitro changes in each participant's cells when exposed to resveratrol in laboratory culture.
- Describe the most feasible methods and standardised outcome measures for future trials.
- Describe features of the research infrastructure which optimised recruitment, retention and communication with participants and families.
- Describe participants' and their families' perceptions of research and important potential barriers to participation.

Methods

Study design and participants

CARDIOlipin MANipulation trial (CARDIOMAN) was a double-blind, placebo-controlled crossover randomised trial. Treatment was given in two 15-week periods with a minimum of 1 month's washout period between these when no treatment was given.

Twenty-six males and one female with the disease were alive in the UK when the trial was designed. Males aged ≥ 6 years in the UK diagnosed with Barth syndrome were eligible. Exclusion criteria were: hypersensitivity to bezafibrate or component of the investigational medicinal product (IMP), photoallergic or phototoxic reaction to fibrate, unstable cardiac condition, cardiac chamber shortening fraction < 25% or significant decrease in shortening fraction in the preceding year, atrial or ventricular arrhythmia not stabilised by treatment, inability to swallow the IMP, hepatic dysfunction, renal impairment (creatinine clearance < 90 ml/min), gallbladder disease, recent unspecified significant deterioration in general health, prisoners and adults lacking capacity to provide informed consent.

Randomisation and masking

Participants were randomised to receive either bezafibrate then placebo, or the reverse order. Allocations were generated in advance by the Bristol Trials Centre and provided to the trial pharmacy which dispensed the IMP. All investigators, members of the research team (apart from pharmacy) and participants remained blind to treatment allocation.

Intervention and procedures

The intervention IMP was bezafibrate taken orally as 100-mg tablets. Children aged 6–9 years started on 100 mg once daily for the first 4 weeks and, if well tolerated, increased to 100 mg twice daily for the remaining 11 weeks. Children aged 10–17 years started on 200 mg once daily for the first 4 weeks and, if well tolerated, increased to 200 mg twice daily for the remaining 11 weeks. Adults (≥ 18 years) took 200 mg twice daily throughout.

The comparator IMP was placebo taken orally as visually identical tablets and manufactured to be as similar as possible in taste and smell to the intervention. The IMP was prescribed at the start of the first period and again at the end of the first period for the second period.

Outcomes

All in vivo outcomes except magnetic resonance spectroscopy (MRS) were assessed three times: at baseline and in the final week of each treatment phase. The primary outcome measure was peak oxygen consumption on bicycle ergometry [peak volume of oxygen (VO_2)]. Secondary outcomes were: MLCL/L4-CL ratio and cardiolipin profile in blood cells; phosphocreatine (PCr)/adenosine 5' triphosphate (ATP) ratio in cardiac muscle on ^{31}P MRS; skeletal muscle oxidative function/on ^{31}P MRS; QoL [age-appropriate Pediatric Quality of Life Inventory (PedsQL™) questionnaires]; absolute neutrophil count; amino acid expression (serum arginine and cysteine); cardiac function [left and right ventricular function: left ventricular ejection fraction (LVEF%), right ventricular ejection fraction (RVEF%); and 2-D longitudinal and circumferential strain at rest, peak exercise and after recovery]; mitochondrial size and number in lymphocytes; mitochondrial area in lymphocytes and as a proportion of cytoplasm area; mitochondrial function and cristae organisation in lymphocytes/neutrophils; arrhythmia profile from 12-lead electrocardiogram at rest and during exercise. Qualitative research methods explored participants' and families' experiences of the different interventions.

Adverse events

Serious adverse events (SAEs) and non-serious adverse events (AEs) were collected from participants from the time of consent until 1 month after the final period. Participants' general practitioners were asked to inform the research team of suspected AEs. Participants were asked questions about SAEs/AEs at each research clinic visit and in monthly phone calls, including during the washout period. SAEs were reviewed by the Chief Investigator, who decided whether the SAE was related to bezafibrate.

Statistical analysis

A total of 20 males aged ≥ 6 years were potentially eligible. The sample size was dictated by the number of eligible boys willing to take part. The primary analysis estimated the difference in mean peak VO_2 between placebo and bezafibrate periods (two-tailed 5% significance level). A sample size of 12 participants allowed the trial to detect a difference of 0.90 (within subject) standard deviations with 80% power.

The statistical analysis plan (SAP) prespecified that all analyses should be conducted according to the intention-to-treat (ITT) principle. The ITT population comprised all participants and periods, according to the randomised allocations. The safety population comprised all randomised participants, according to the treatment received in each period, who received at least one dose of IMP.

All statistical tests were two-sided and used a 5% significance level, except for interactions that used a 10% significance level. Treatment effects were estimated with 95% confidence intervals (CIs). No formal adjustment was made for multiple testing.

The treatment effect for the primary outcome was analysed using mixed linear regression models, adjusting for period (fixed effect; exploring interactions where necessary) and participants (random effects). Model assumptions were tested using standard methods, and the carry-over effect was estimated by including treatment order. Treatment effects for continuously scaled secondary outcomes (prespecified in the SAP) were estimated and tested using the same methods.

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Results

There was no statistically significant difference between bezafibrate and placebo in peak VO_2, which was 0.66 ml/kg/min lower (95% CI −2.34 to 1.03; p = 0.43) with bezafibrate.

There was a borderline statistically significant increase in LVEF% using echocardiography (3.72%, 95% CI −0.26% to 7.69%; p = 0.065) but no evidence of a difference when using magnetic resonance imaging (MRI) (0.15%, 95% CI −3.21% to 3.51%; p = 0.926). There was also no difference for RVEF% between bezafibrate and placebo (0.32, 95% CI −2.32 to 2.96; p = 0.801). Longitudinal and circumferential strain at rest were better (lower) with bezafibrate than with placebo; differences were −1.67 (95% CI −3.11 to −0.22; p = 0.026) and −2.72 (95% CI −5.03 to −0.40; p = 0.024) respectively. There was no difference in diastolic ratio at rest between bezafibrate and placebo, and no differences in strain or diastolic ratio at peak exercise or after 2 minutes' recovery.

All participants had sinus rhythm at rest and during exercise for all three time points.

Magnetic resonance spectroscopy was only performed after the second period for 10 participants. PCr/ATP ratio in cardiac muscle was not collected. Cross-sectionally (five vs. five participants), MRS appeared to increase Tau and reduce Q_{max} parameters with bezafibrate compared to placebo.

Monolysocardiolipin/L4-CL ratio did not differ between bezafibrate and placebo but deteriorated in the second period (20% higher, 95% CI 3% to 40%; p = 0.024). Neutrophil count also showed no difference between bezafibrate and placebo but increased in the second period (2.26, 95% CI 0.08 to 4.45; p = 0.043). Plasma cysteine increased with bezafibrate (32% higher, 95% CI 8% to 62%; p = 0.001) but plasma arginine did not (25% higher, 95% CI −10% to 72%; p = 0.180). Mitochondrial outcomes on blood samples did not show marked differences and were not formally compared.

Quality-of-life scores were transformed to a 0–100 scale (higher being better). Bezafibrate had no effect on core (−3.41, 95% CI −8.67 to 1.86; p = 0.192) or fatigue (1.71, 95% CI −6.28 to 9.71; p = 0.659) QoL scores.

Emerging themes identified in qualitative interviews were classified as overarching, related to lived experience with Barth syndrome, and trial participation and conduct. Participants and families were pleased to take part in the trial, viewing participation as 'giving back' to the medical community. No participant withdrew, and the trial was not perceived to be too onerous for families. Participating in the research gave both individuals and families a sense of responsibility for their condition, partly driven by appreciation for the Barth Syndrome Service and staff expertise.

Monolysocardiolipin/L4-CL ratio in vitro was estimated from samples of cells incubated with control, bezafibrate or resveratrol. Each drug reduced the MLCL/L4-CL ratio, largely by increasing intermediate cardiolipin species, and more so with resveratrol than with bezafibrate. Ratios were nevertheless profoundly abnormal in comparison to patients with intermediate Barth phenotypes.

Discussion

Main findings: study results

There was no clinically important or statistically significant improvement in peak VO_2 with bezafibrate compared to placebo.

There were inconsistent findings for LVEF% by method of measurement; echocardiography showed a borderline improvement that cardiac MRI did not demonstrate. Longitudinal and circumferential strain measurements were better at rest with bezafibrate, but not at peak exercise.

There were no clinically important or statistically significant improvements in core or fatigue QoL domains.

Plasma cysteine was significantly improved with bezafibrate treatment. Plasma arginine also increased but not significantly. Neutrophil count was unaffected and there was no significant change in cardiolipin ratio in participants' blood with bezafibrate.

There were no significant period effects except for MLCL/L4-CL ratio and neutrophil count, both increased in the second period.

In vitro incubation of Epstein–Barr virus-transformed patient lymphoblasts with either bezafibrate or resveratrol resulted in improved MLCL/L4-CL ratios, an effect that was more profound with resveratrol. Treatment with this drug also resulted in a more obvious increase in intermediate MLCL species.

Strengths and limitations

Strengths of the trial are: randomisation and placebo blinding; crossover design; excellent retention; completeness of the data, good adherence and IMP tolerance.

There were several limitations. The sample size (outside our control) was very small, and interpretation of effects for many secondary outcomes was challenging.

Longer treatment duration or a higher bezafibrate dose might have produced better outcomes.

Bezafibrate may have induced adverse reactions in some participants, inadvertently causing unblinding.

The group of participants randomised to receive bezafibrate first was older than the group randomised to the opposite order, which may explain period effects.

The electron microscopic and mitochondrial assessments proved problematic and were not completed in vitro. Measurements of mitochondrial content and membrane potential were highly variable.

Amino acid levels were not assayed after fasting.

Echocardiography images during exercise testing were often suboptimal.

Lessons for the future

Magnetic resonance spectroscopy in children is still exploratory, and protocols need to be better validated, but it is considered very promising as a potential objective functional outcome.

Cardiolipin profile is not a good outcome for research, although it is appropriate for diagnosis because the Barth cardiolipin profile is so extremely different from a normal profile.

Electron microscopy measurements of mitochondria should not be considered as outcomes in future trials.

Peak VO_2 should be the preferred current primary outcome because it is resistant to bias compared to other functional outcomes.

Conclusion

The trial did not show a significant treatment effect for the primary outcome, peak VO_2. Some secondary outcomes such as systolic function measured by echocardiography and amino acid levels provided some

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evidence of benefit. The trial provided insights about trial methods and conduct in the context of a very rare paediatric disease that should inform future studies.

Trial registration

This trial is registered as ISRCTN58006579.

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