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ORIGINAL COMMUNICATION

An open-label trial in Friedreich ataxia suggests clinical benefit with high-dose resveratrol, without effect on frataxin levels

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Abstract Friedreich ataxia (FRDA) is due to a triplet repeat expansion in *FXN*, resulting in deficiency of the mitochondrial protein frataxin. Resveratrol is a naturally occurring polyphenol, identified to increase frataxin expression in cellular and mouse models of FRDA and has anti-oxidant properties. This open-label, non-randomized trial evaluated the effect of two different doses of resveratrol on peripheral blood mononuclear cell (PBMC) frataxin levels over a 12-week period in individuals with FRDA. Secondary outcome measures included PMBC *FXN* mRNA, oxidative stress markers, and clinical measures of disease severity. Safety and tolerability were studied. Twenty-four participants completed the study; 12 received low-dose resveratrol (1 g daily) and 12 high-dose resveratrol (5 g daily). PBMC frataxin levels did not change in either dosage group [low-dose group change: 0.08 pg/μg protein (95 % CI −0.05, 0.21, $p = 0.21$); high-dose group change: 0.03 pg/μg protein (95 % CI −0.10, 0.15, $p = 0.62$)]. Improvement in neurologic function was evident in the high-dose group [change in Friedreich Ataxia Rating Scale −3.4 points, 95 % CI (−6.6, −0.3), $p = 0.036$], but not the low-dose group. Significant improvements in audiologic and speech measures, and in the oxidative stress marker plasma F_2-isoprostane were demonstrated in the high-dose group only. There were no improvements in cardiac measures or patient-reported outcome measures. No serious adverse events were recorded. Gastrointestinal side-effects were a common, dose-related adverse event. This open-label study shows no effect of resveratrol on frataxin levels in FRDA, but suggests that independent positive clinical and biologic effects of high-dose resveratrol may exist. Further assessment of efficacy is warranted in a randomized placebo-controlled trial.

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Introduction

Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disorder characterized by progressive ataxia, lower limb areflexia, extensor plantar responses, dysarthria, diminished posterior column function, and weakness. Scoliosis, auditory neuropathy, and a cardiomyopathy are also common [1].

More than 90 % of individuals with FRDA are homozygous for a GAA triplet repeat expansion in the first intron of *FXN* [1, 2]. *FXN* encodes the mitochondrial protein frataxin, whose expression is markedly reduced in individuals with FRDA [3]. Frataxin deficiency leads to impaired cellular iron homeostasis and impaired synthesis of iron-sulfur cluster-containing proteins, leading to mitochondrial iron accumulation, impaired oxidative phosphorylation, and oxidative stress [4]. Frataxin levels are also reduced in asymptomatic carriers, [5] indicating that even a moderate increase in frataxin expression should provide therapeutic benefit.

Resveratrol is a naturally occurring polyphenol found in red wine and other edible sources. It is postulated to have wide-ranging health benefits, including antioxidant, anti-carcinogenic, anti-diabetic, and neuroprotective properties. Importantly, for clinical applications, resveratrol has a good safety profile [6, 7].

Resveratrol increases frataxin expression in both in vitro and in vivo models of FRDA. A 1.5- to 2-fold increase in frataxin protein expression was observed in lymphoblasts and fibroblasts derived from individuals with FRDA, [8] and 200 mg/kg subcutaneous resveratrol resulted in a 1.5-fold increase in human frataxin protein in the brain of humanized FRDA (YG8R) mice [8]. These findings, in combination with resveratrol's antioxidant and neuroprotective properties and good safety profile, led to the design of this open-label trial of resveratrol in individuals with FRDA.

Methods

Study design

This was an open-label, non-randomized, proof-of-principle study evaluating the efficacy and safety of two different doses of resveratrol over a 12-week treatment period in individuals with FRDA. The primary outcome measure was peripheral blood mononuclear cell (PBMC) frataxin levels at 12 weeks. Secondary outcome measures included PBMC *FXN* mRNA levels, oxidative stress markers, ataxia rating scales, clinical measures of speech and audiologic function, echocardiographic variables, and quality of life at 12 weeks. Safety and tolerability, and first-dose pharmacokinetic data were also studied.

Participants were assigned to either a low-dose or high-dose treatment arm (Fig. 1). There was no formal randomization process. The low-dose group received resveratrol 0.5 g twice daily (b.i.d.) (99.5 % pure trans-resveratrol, 500 mg capsules, Megaresveratrol, Danbury, CT). The high-dose group received resveratrol 2.5 g b.i.d. Enrolment of participants into the high-dose group (Stage 2) commenced after the Data Safety Monitoring Committee had reviewed adverse events in the low-dose group treated for at least 4 weeks.

Inclusion criteria included age > 18 years, genetically confirmed FRDA due to homozygosity for the *FXN* GAA triplet repeat expansion, a score of ≥ 1 on the ataxia subscale of the Friedreich Ataxia Rating Scale (FARS) [9], and adequate end organ function. Exclusion criteria included recent non-elective hospitalization, pregnant/lactating women, unwillingness to practice contraception during the study, active arrhythmias and/or cardiac insufficiency, or prior invasive cancer. Because of the potential for CYP450-related drug interactions [10], the use of medications with significant CYP450 metabolism and narrow therapeutic indices (e.g. amiodarone, warfarin) was exclusionary. Participants taking idebenone, vitamin E, coenzyme Q_{10}, or other antioxidants (including ascorbic acid) underwent a 30-day washout prior to enrolment.

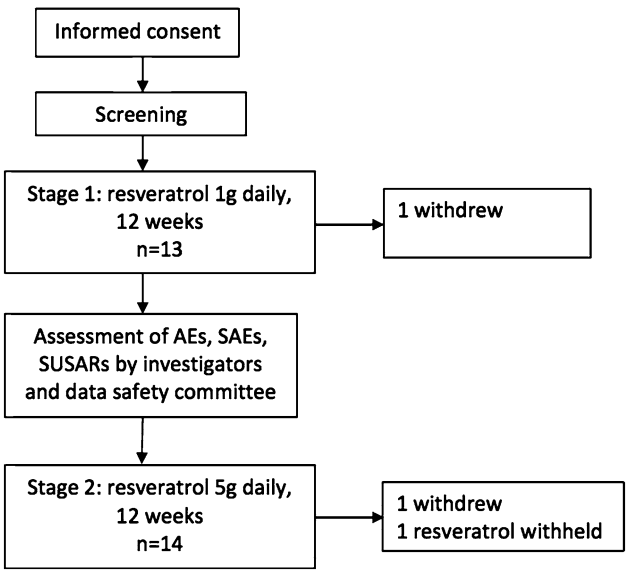

Fig. 1 Study design. *AE* adverse event, *SAE* serious adverse event, *SUSAR* suspected unexpected serious adverse reaction

Study conduct

Participants attended a screening visit to confirm eligibility. Biomarker and clinical assessments were undertaken at baseline (day 1) and week 12 (end of study) visits. After completion of baseline assessments, participants were administered the first dose of resveratrol (0.5 or 2.5 g) after a standardized low-fat breakfast (containing <20 g of fat) to minimize variability in pharmacokinetic parameters [11].

Safety assessments were performed at weeks 1, 2, 3, 4, 6, 8, and 12, and included review of adverse events, a brief physical examination, and monitoring of blood hematology and biochemistry. Careful monitoring for potential renal complications was undertaken due to the occurrence of cast nephropathy in a multiple myeloma trial of SRT501 (micronized resveratrol) [12].

Outcome measures

Primary outcome measure

PBMC frataxin levels were measured at baseline and week 12 as previously described [13]. Levels were normalized to the protein content in each sample assessed by Fourier transform infrared (FT-IR)-based method using the Direct Detect® spectrometer (Millipore, Austria).

Secondary outcome measures

Secondary outcome measures were assessed at baseline and week 12. *FXN* mRNA levels were measured in PBMCs as previously described [14]. The oxidative stress markers plasma F_2-isoprostane (a marker of lipid peroxidation) [15] and urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) [16] levels were quantified as previously described. Neurologic impairment was assessed with three ataxia rating scales: FARS [9], International Cooperative Ataxia Rating Scale (ICARS) [17], and Scale for the Assessment and Rating of Ataxia (SARA) [18]. The individual and composite components of the Friedreich Ataxia Composite Test were also administered [19]. Open-set speech perception was assessed in one ear using a 50-word consonant-nucleus-consonant (CNC) word list with a competing noise at a signal-to-noise ratio of 0 dB [20]. Dysarthria was assessed using quantitative data extracted from speech recordings of four speech tasks (reading a phonetically balanced paragraph, producing a sustained vowel sound ('Aaah') for 5 s, saying the days of the week, and producing a 1 minute monolog with positive content) [21]. Quality of life and disease impact were assessed using the Friedreich Ataxia Impact Scale (FAIS) [22] and SF36-version 2 [23]. Echocardiographic images were obtained and off-line measurements were performed using a standardized protocol which included M-mode, 2-D and pulsed wave Doppler imaging as previously described [24]. Assessors of clinical measures were not blinded to the visit type (baseline versus final visit), and for some measures, the dosage group of the participant.

First-dose pharmacokinetic characterization of resveratrol and its sulfate and glucuronide conjugates was performed. Samples were obtained at baseline, 45 and 90 min post dose, and prepared and analyzed based on a previously reported method [25].

Standard protocol approvals, registrations, and patient consents

This study was approved by the Human Research and Ethics Committee, Monash Health (Reference number 10358B). Written informed consent was obtained from all participants. This trial was registered on clinicaltrials.gov (NCT01339884).

Statistical analysis

Primary data analysis was performed on data from participants who completed the 12-week trial. A secondary 'intention-to-treat' analysis of data from all enrolled participants was also performed. For each dosage group, the mean absolute change from baseline to 12 weeks in all outcome measures was calculated and presented along with its 95 % confidence interval (CI). Paired t tests were used to test the null hypothesis of no absolute change in each of these parameters from baseline to 12 weeks, carried out separately for each dosage group. A $\log_{10}$ transformation was used for mean silence length. The absolute change in urinary 8-OHdG levels was assessed using the Wilcoxon signed-rank test, as this data was not normally distributed. Adverse events were documented in all enrolled participants.

Statistical analysis was performed using Stata statistical software 12.1 software (Stata Corp., 2013, College Station, TX, USA).

Results

A total of 27 participants were enrolled in the study—13 in the low-dose group and 14 in the high-dose group. Three participants did not complete the 12-week study: two (one in each dosage group) discontinued due to non-serious adverse events, and one (in the high-dose group) had the study drug withheld for 5 weeks during investigation of what was subsequently deemed to be spurious laboratory monitoring results. The primary analysis was, therefore, conducted on 24 participants—12 in each dosage arm.

Baseline participant characteristics of the 24 participants who completed the study are summarized in Table 1.

Primary outcome measure

There was little change in PBMC frataxin levels from baseline to 12 weeks after low-dose [mean change in frataxin: 0.08 pg/μg protein (95 % CI −0.05, 0.21, $p = 0.21$)] or high-dose resveratrol treatment [mean change in frataxin: 0.03 pg/μg protein (95 % CI −0.10, 0.15, $p = 0.62$)] (Table 2).

Secondary outcome measures

The results of the secondary outcome measures are summarized in Tables 2 and 3.

Biomarkers

Levels of *FXN* mRNA were similar after 12 weeks of treatment with either low- or high-dose resveratrol. The oxidative stress marker plasma F_2-isoprostane decreased in participants receiving high-dose resveratrol for 12 weeks [−216.8 pmol/L plasma (95 % CI −301.4, −132.2, $p < 0.001$)]; however, levels remained similar in participants receiving low-dose resveratrol. Urinary 8-OHdG concentrations were similar after 12 weeks of treatment in both dosage groups.

Clinical outcome measures

There was an improvement in neurologic deficit after 12 weeks of treatment in participants receiving high-dose resveratrol as measured by the FARS [change in score −3.4 points, 95 % CI (−6.6, −0.3), $p = 0.036$] and ICARS [change in score −1.9 points, 95 % CI (−3.1, −0.8), $p = 0.004$]. Improvements were seen predominantly in the 'Neurological Examination' subscale of the FARS (upper limb and bulbar components), and the 'Posture' subscale of the ICARS (data not shown). There was also a trend for improvement in the SARA, but little evidence of improvement in components of the Friedreich Ataxia Composite Test.

There was evidence of an improvement in speech perception in background noise in the high-dose resveratrol group [improvement in 'percentage phonemes correct' of 4.6 %, 95 % CI (1.0, 8.2), $p = 0.02$]. The speech variable mean silence length was reduced in two out of three speech tasks. This finding, in combination with a trend to a reduction in percentage silence time in the same speech tasks reflects improved speech efficiency. There was little evidence of improvement in speech rate, pitch control or voice quality (data not shown).

There was little evidence of improvement in any neurologic, audiologic or speech outcome measures in individuals treated with low-dose resveratrol. There were no significant changes in any components of the FAIS or SF-36 in either dosage group (Supplementary data).

Three participants had a reduced left ventricular ejection fraction at baseline (<50 %). Echocardiographic measures were similar at baseline and 12 weeks in both dosage groups, including left ventricular end-diastolic diameter, left ventricular mass index, relative wall thickness, ejection fraction, and tissue Doppler mitral annular early diastolic velocities (Supplementary data).

Safety and tolerability

Table 4 summarizes adverse events. No serious adverse events were observed during the study. Resveratrol at a dose of 1 g daily was generally well tolerated, apart from one subject who withdrew due to fatigue.

Diarrhea and loose stools were frequent in individuals receiving high-dose resveratrol, occurring in 10 (71 %) and 12 (86 %) individuals, respectively. Diarrhea was mild in severity in one, moderate in seven, and severe in two participants. Diarrhea/loose stools were generally noted within a few days of study drug commencement and

Table 1 Baseline characteristics of 24 participants who completed the trial

Characteristic	1 g daily ($n = 12$)	5 g daily ($n = 12$)
Age, years, mean (SD)	34.9 (12.7)	39.2 (7.7)
Age at onset, years, mean (SD)	15.6 (6.1)	19.7 (7.7)
Disease duration, mean (SD)	19.3 (13.4)	19.4 (6.7)
GAA1 repeat length, mean (SD)	624 (171)	568 (212)
Male, number (%)	7 (58.3)	9 (75.0)
Baseline FARS score, mean (SD)	98.5 (27.2)	91.8 (26.0)
Baseline ICARS score, mean (SD)	51.9 (17.2)	49.1 (17.5)

SD standard deviation, *GAA*1 size of the smaller GAA *FXN* repeat, *FARS* Friedreich Ataxia Rating Scale (167-point version), *ICARS* International Cooperative Ataxia Rating Scale

Table 2 Biomarker results in low- and high-dose resveratrol treatment groups

Resveratrol dose			1 g daily ($n = 12$)			5 g daily ($n = 12$)		
Outcome measure	Baseline mean (SD)	Final mean (SD)	Mean difference (95 % CI)[a]	p value[a]	Baseline mean (SD)	Final mean (SD)	Mean difference (95 % CI)[a]	p value[a]
Frataxin (pg/μg protein)	0.37 (0.15)	0.45 (0.24)	0.08 (−0.5, 0.21)	0.21	0.40 (0.18)	0.42 (0.21)	0.03 (−0.10, 0.15)	0.62
FXN mRNA (% relative *FXN* expression)	20.5 (4.9)	21.8 (6.8)	1.3 (−0.9, 3.6)	0.22	25.8 (11.2)	23.6 (11.6)	−2.2 (−4.7, 0.3)	0.08
Plasma F_2-isoprostane (pmol/L plasma)	1332.8 (239.0)	1287.6 (233.0)	−45.2 (−152.9, 62.6)	0.38	1465.3 (392.8)	1248.4 (333.6)	−216.8 (−301.4, −132.2)	**<0.001**
Urinary 8-OHdG (ng/mg creatinine)[b]	7.5 (10.4)	4.8 (3.6)	–	0.24[c]	2.9 (5.2)	2.4 (4.1)	–	0.31[c]

p value less than 0.05 is in bold

8OHdG urinary 8-hydroxy-2′-deoxyguanosine, *CI* confidence interval, *SD* standard deviation

[a] Mean difference and *p* values are paired *t* test statistics unless otherwise stated. Mean difference is (final − baseline) values

[b] Values presented as median (IQR)

[c] Wilcoxon signed-rank test *p* value

resolved within a few days of study completion. Only two participants did not report this side-effect; however, both experienced abdominal pain. Abdominal pain, nausea and flatulence were common in the high-dose group. Seven participants required symptomatic treatment of diarrhea with loperamide. Four individuals in the high-dose group required dosage reduction: two to 2 g resveratrol daily, and two to 4 g daily.

One subject in the high-dose group developed cholestatic liver function abnormalities within 4 weeks of study commencement. Gamma glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) peaked at 10- and 4-times the upper limit of normal (ULN), respectively, and were associated with moderate nausea and abdominal pain. Transaminases remained below twice the ULN, and hepatic synthetic function was preserved. The drug was ceased after 28 days and the subject discontinued the study. An extensive work-up for infectious, immunologic, and genetic causes of liver dysfunction was normal. Liver function normalized within 8 weeks of drug cessation.

First-dose pharmacokinetics

Plasma concentrations of resveratrol and four metabolites are summarized in Table 5. At 90 min, the mean plasma concentrations of resveratrol were 127.7 (SD 101.1) ng/mL and 261.1 (SD 241.7) ng/mL after a single dose of 0.5 and 2.5 g resveratrol, respectively. The mean difference between these two doses was 133.4 ng/mL (95 % CI −15.6, 282.4 ng/mL) with an one-sided p value of 0.039.

Secondary analysis

A similar pattern of results was seen from the intention-to-treat analysis of data from all 27 enrolled participants (Supplementary data).

Discussion

In this open-label trial of resveratrol in FRDA we found little change in the primary outcome measure, PBMC frataxin protein levels, after 12 weeks of treatment with either low- (1 g daily) or high-dose (5 g daily) resveratrol. However, treatment with high-dose resveratrol resulted in improvement in both oxidative stress (as measured by plasma F_2-isoprostane levels) and some clinical outcome measures including neurologic, audiologic, and speech function.

Although this trial was open-label and a placebo effect cannot be excluded, the improvement in a biologic marker and more than one independent clinical outcome measure in the high-dose group, in the absence of significant

Table 3 Clinical outcome measures in low- and high-dose resveratrol treatment groups

Resveratrol dose	1 g daily ($n = 12$)				5 g daily ($n = 12$)			
Outcome measure	Baseline mean (SD)	Final mean (SD)	Mean difference (95 % CI)	p value	Baseline mean (SD)	Final mean (SD)	Mean difference (95 % CI)	p value
Total FARS score	98.5 (27.2)	95.9 (23.2)	−2.7 (−6.8, 1.4)	0.17	91.8 (26.0)	88.4 (25.2)	−3.4 (−6.6, −0.3)	**0.036**
Total ICARS score	51.9 (17.2)	51.6 (17.5)	−0.3 (−3.2, 2.6)	0.80	49.1 (17.5)	47.2 (17.5)	−1.9 (−3.1, −0.8)	**0.004**
Total SARA score	21.9 (8.9)	21.6 (8.3)	−0.3 (−1.8, 1.3)	0.73	20.5 (7.9)	19.6 (8.2)	−1.0 (−2.1, 0.1)	0.08
LCLA	120.3 (36.5)	120.8 (35.5)	0.4 (−2.9, 3.8)	0.79	116.9 (39.2)	119.0 (39.5)	2.1 (−1.3, 5.5)	0.20
T25FW, sec[a]	7.2 (2.2)	7.5 (2.0)	0.4 (−0.7, 1.4)	0.38	7.7 (3.2)	9.8 (7.9)	2.0 (−4.0, 8.0)	0.40
9HPT, sec[b]	70.9 (16.3)	71.4 (19.4)	0.6 (−4.9, 6.0)	0.82	52.2 (10.7)	52.8 (15.3)	0.6 (−4.7, 5.9)	0.79
FACT Z2	0.023 (0.80)	0.003 (0.78)	−0.020 (−0.075, 0.035)	0.44	0.22 (0.80)	0.22 (0.81)	0.002 (−0.074, 0.077)	0.96
FACT Z3	0.086 (0.78)	0.077 (0.76)	−0.010 (−0.063, 0.043)	0.69	0.19 (0.81)	0.21 (0.81)	0.019 (−0.036, 0.075)	0.46
Phoneme score[c]	45.0 (21.2)	46.2 (20.4)	1.3 (−2.3, 4.9)	0.45	50.2 (18.9)	54.8 (18.7)	4.6 (1.0, 8.2)	**0.02**
Days of the week								
Log_{10} mean silence length	−1.32 (0.19)	−1.40 (0.24)	−0.08 (−0.20, 0.05)	0.21	−1.39 (0.16)	−1.52 (0.18)	−0.13 (−0.22, −0.04)	**0.007**
Percent silence	11.3 (7.5)	11.6 (8.7)	0.3 (−4.7, 5.2)	0.91	10.9 (6.1)	7.8 (5.5)	−3.0 (−6.4, 0.3)	0.072
Free passage[d]								
Log_{10} mean silence length	0.91 (0.21)	0.93 (0.21)	−0.01 (−0.10, 0.08)	0.75	0.91 (0.20)	−1.05 (0.13)	−0.14 (−0.25, −0.03)	**0.017**
Percent silence	28.4 (9.0)	28.0 (9.2)	−0.4 (−6.3, 5.6)	0.90	27.5 (11.2)	22.3 (8.9)	−5.2 (−10.7, 0.4)	0.067

Mean difference and p values are paired t test statistics. Mean difference is (final − baseline) values

p values less than 0.05 are in bold

CI confidence interval, *SD* standard deviation, *FARS* Friedreich Ataxia Rating Scale (167-point version), *ICARS* International Cooperative Ataxia Rating Scale, *SARA* Scale for the Assessment and Rating of Ataxia, *LCLA* Sloan low contrast letter acuity test, *T25FW* Timed 25-foot walk, *9HPT* 9-hole pegboard test; averaged between both hands, *FACT* Freidreich Ataxia Composite Test, *Z2* composite of *Z* scores from $9HPT^{-1}$ and $T25FW^{-1}$, *Z3* composite of *Z* scores from $9HPT^{-1}$, $T25FW^{-1}$ and LCLA, *Phoneme score* percentage phonemes correct in 50-word speech perception test in background noise, *Days of the week* speech task, *Free passage* speech task

[a] 5 out of 12 participants completed the T25FW in each dosage group

[b] 10 out of 12 participants completed the 9HPT in each dosage group

[c] 11 out of 12 participants completed audiology testing in the low-dose group

[d] 11 out of 12 participants completed the free passage speech task in the low-dose group

Table 4 Adverse events in all enrolled participants

Adverse event, *n* (%)	1 g daily ($n = 13$)	5 g daily ($n = 14$)
Infections		
Upper respiratory tract infection	4 (31)	5 (36)
Urinary tract infection	3 (23)	1 (7)
Sinusitis	1 (8)	0
Tonsillitis	1 (8)	0
Nervous system disorders		
Headache	4 (31)	3 (21)
Fatigue	3 (23)	2 (14)
Gastrointestinal disorders		
Loose stools	1 (8)	12 (86)
Diarrhea	1 (8)	10 (71)
Abdominal pain/cramps	2 (15)	10 (71)
Nausea	1 (8)	5 (36)
Bloating	1 (8)	1 (7)
Flatulence	0	2 (13)
Constipation	0	1 (7)
Dyspepsia	1 (8)	1 (7)
Abnormal liver function tests[a]	1 (8)	1 (7)
Cardiac disorders		
Palpitations	1 (8)	0
Increased creatine kinase level	2 (15)	2 (14)
Renal disorders		
Microalbuminuria	1 (8)	3 (21)
Dermatologic disorders		
Skin rash	0	4 (29)
Lower limb oedema	0	1 (7)

[a] Liver enzymes raised to greater than twice the upper limit of normal

changes in the low-dose group, represents an important finding. This raises a number of questions as to how resveratrol may produce downstream clinical and biologic improvements in FRDA in the absence of a demonstrable change in frataxin levels in PBMCs.

In humanized FRDA (YG8R) mice treated with resveratrol, frataxin protein and *FXN* mRNA levels were quantified in brain tissue, a site of primary pathology in FRDA [8]. Whilst PBMC frataxin levels correlate with skeletal muscle levels in humans [26], an increase in frataxin in the central nervous system in the absence of detectable changes in PBMCs is possible and may explain the findings in this study.

The doses of resveratrol utilized for in vitro animal and human studies vary enormously [7]. The optimal dosage of resveratrol has not been determined. To add to the complexity, dose–response relationships are not always linear [27] and may vary according to the tissue and/or biologic target in question [6]. The poor oral bioavailability and rapid metabolism of resveratrol [7] also present important pharmacokinetic limitations that may impact tissue concentrations and, therefore, translation into clinical benefit. First-dose pharmacokinetics in the present study indicate that plasma concentrations achieved were approximately an order of magnitude lower than those used in vitro by Li et al. [8]. Although the human equivalent dose of 200 mg/kg resveratrol administered subcutaneously to YG8R mice [8] (16.2 mg/kg, i.e. 1135 mg for a 70 kg adult, based on body surface area normalization) is comparable to the doses used in this trial, the subcutaneous administration in YG8R mice would result in higher plasma and tissue resveratrol concentrations than the oral route used in the current study.

The neurologic, audiologic, and speech improvements documented in the high-dose group may be attributed to placebo or practice effects. Plasma F_2-isoprostane levels are an objective outcome measure, however, and the speech outcome measures have demonstrated resistance to practice effects [21]. Placebo and practice effects would also be expected to be similar in both dosage groups. Given that responsiveness to change of the FARS and ICARS can vary with clinical or genetic factors [28], we compared baseline parameters between the low- and high-dose groups and confirmed that GAA1 size (size of the smaller GAA *FXN* repeat), baseline FARS/ICARS, and disease duration were similar in the two groups. Compared to natural history

Table 5 First-dose pharmacokinetic data for all enrolled participants

Metabolite (ng/mL)	0.5 g single dose ($n = 13$)		2.5 g single dose ($n = 14$)	
	45 min	90 min	45 min	90 min
Resveratrol[a]	#	127.7 (101.1)	72.5 (110.1)	261.1 (241.7)
Resveratrol-3-glucuronide[b]	2.5 (45.6)	349 (1106)	185 (314)	1965 (1851)
Resveratrol-4′-glucuronide[b]	3.9 (60)	298 (1336)	159 (271)	1645 (1644)
Resveratrol-3-sulfate[b]	4.9 (78)	674 (887)	183 (354)	1173 (2003)
Resveratrol-4′-sulfate[b]	*	7.1 (8.5)	0 (2.4)	11.5 (13.5)

[a] Values presented as mean (SD)

[b] Values presented as median (IQR)

10/13 had levels below lower limit of detection or quantification

* 11/13 had levels below lower limit of detection or quantification

studies of FRDA over 12 months [29, 30], the improvement in FARS and ICARS in the high-dose group is of a clinically relevant magnitude. The absence of any change in cardiac measures may be due to true lack of cardiac benefit from resveratrol, but could also reflect the short trial duration.

The reduction in plasma F2-isoprostane levels in the absence of changes in urinary 8-OHdG levels in the high-dose resveratrol group confirms the antioxidant activity of resveratrol, at least on lipid peroxidation. Studies of oxidative stress in FRDA have provided conflicting findings [4] and highlight the potential limitations of these measures. Notably, plasma/urine levels of oxidative stress markers may not reflect levels observed in other body or cell compartments, in particular the central nervous system.

There are a number of potential mechanisms for a therapeutic effect of resveratrol in FRDA. Resveratrol activates SIRT1, a NAD+-dependent deacetylase [27, 31], which activates the transcriptional coactivator PGC-1α [32], thought to play an important role in the pathogenesis of FRDA [33, 34]. Downstream transcription targets of PGC-1α include genes involved in mitochondrial biogenesis [32] and antioxidant defenses [35]. Downregulation of PGC-1α is reported in FRDA, and thought to be responsible, at least in part, for the reduced antioxidant response seen. Pharmacologic activation of PGC-1α was reported to increase PGC-1α and SOD2 levels in the absence of any effect on frataxin expression, an interesting finding given the results of our open-label trial [35]. Resveratrol may also enhance antioxidant responses by activating the human homolog of nuclear factor erythroid 2-related factor 2, a key transcription factor in cellular antioxidant activity, as observed in rats [36]. High-dose resveratrol may, therefore, activate antioxidant rather than mitochondriogenic pathways in individuals in FRDA.

No serious adverse events were recorded during this 12-week study. Low-dose resveratrol was generally well tolerated. Gastrointestinal side-effects were frequent in participants receiving 5 g resveratrol daily, limiting tolerability. Diarrhea appeared dose-related: it occurred to a moderate to severe degree in 70 % in the high-dose group, consistent with other trials administering daily doses of greater than 2 g/day of resveratrol [11, 37]. Its mechanism is unclear and may be related to resveratrol, its metabolites, or unabsorbed components of the formulation in the gastrointestinal tract [38]. Clinically significant abnormalities in liver function were documented in one subject. The mechanism for this is unknown and has implications for safety monitoring in individuals taking high-dose resveratrol.

Despite no improvement in the primary outcome measure, this open-label trial demonstrated improvements in some, but not all, clinical and biologic secondary outcome measures after treatment with high-dose resveratrol. Limitations of this study include its open-label design, and the fact that it was not powered on the secondary endpoints in which improvements were noted. As such, the improvements seen in the high-dose group could be a chance finding given the small number of participants and the number of outcomes that we measured. As such, recommendations regarding the efficacy of resveratrol cannot be made from this open-label study. Nevertheless, we believe that further assessment of the clinical efficacy of resveratrol is warranted in a randomized placebo-controlled setting. Whilst resveratrol was safe, the high frequency of dose-related gastrointestinal side-effects with high-dose resveratrol limits its tolerability and prevents adequate blinding of participants. Using bioequivalent doses micronized resveratrol with three times the bioavailability of standard resveratrol [38] may improve tolerability. Other sirtuin-activating compounds may also provide therapeutic alternatives [31]. A greater understanding of the mechanisms of effect of resveratrol in FRDA is required to determine which therapeutic pathway(s) are best exploited.

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