Safety and tolerability of phenylbutyrate in inclusion body myositis

Duaa Jabari[1], Ali Ciersdorff[2], Andrew Heim[2], Heather Wilkins[2], Abdulbaki Agbas[3], Edina Kosa[3], Suzanne L. Hunt[2], Mamatha Pasnoor[2], Mazen M. Dimachkie[2], Richard J. Barohn[4]

[1] Cedars-Sinai Medical Center, Los Angeles, CA
[2] University of Kansas Medical Center, Kansas City, KS
[3] Kansas City University, Kansas City, MO
[4] University of Missouri School of Medicine, Columbia, MO

ABSTRACT

Introduction

Phenylbutyrate (PBA) showed a positive effect on the muscle cell model of Inclusion Body Myositis (IBM) by improving lysosomal activity, ameliorating consequences of impaired autophagy, and decreasing vacuolization. This provides a rationale to study this medication in patients with IBM.

Objectives

To evaluate the safety and tolerability of phenylbutyrate in IBM and monitor for any early signal of effectiveness.

Methods

Open-label study of 10 subjects with IBM who received treatment with PBA for 3 months after a 3-month run-in period. The PBA dose was 3 gm twice daily. The primary outcome measure was adverse event reporting. Secondary outcome measures included manual muscle testing, timed up and go test, IBM functional rating scale, and grip strength, along with exploratory biomarkers evaluating the mitochondrial function, stress response, degenerative process, and apoptosis.

Results

Ten subjects completed the study. PBA was well tolerated with no serious adverse events related to it. The most common adverse events were gastrointestinal-related and did not require stopping treatment. One of the biomarkers (MitoTracker) showed a statistically significant drop over the treatment period of the study (p-value of 0.02 for the mean change). There were no statistically significant changes in other secondary outcome measures, but the study was limited by a small sample size and short treatment period.

Conclusions

Phenylbutyrate was safe and well tolerated in patients with IBM in this pilot study. The change in the MitoTracker suggests target engagement, but a Phase II study is needed to confirm and study the efficacy of PBA in IBM.

Introduction

Inclusion body myositis (IBM) is the most common acquired muscle disorder after age 50. The prevalence of IBM varies from study to study and ranges in United States from 10.7 per million (28.9 per million for age 45 and older)[1] to 70.6 per million[2] with male to female ratio of 2:1 to 3:1.[3] While IBM does not change life expectancy, it causes significant disability.

IBM was classically classified as inflammatory myopathy considering inflammatory infiltrate found on muscle biopsy. The lack of response to treatment raised the impression that IBM is a degenerative disorder with secondary inflammation.[4] This was further supported by other findings on muscle biopsy like inclusions and amyloid deposits as can be seen in other neurodegenerative disorders.

The abnormal accumulation of amyloid-beta protein precursor, and its proteolytic fragment amyloid-beta, associated with intracellular aging of muscle fibers, is thought to be the key pathogenic event.[5] Low-molecular amyloid-beta oligomers, which are highly cytotoxic, have been demonstrated in IBM muscle tissues with immunoblots but not in control muscle tissue.[6]

Studies have suggested that cultured human muscle fibers (CHMFs) with experimentally inhibited autophagy and lysosomal activity had pronounced vacuolization, in addition to significantly increased amyloid-beta and its oligomers.[7-8]

Many similar neurodegenerative disorders, termed "protein-misfolding disorders" are characterized by the accumulation of intracellular or extracellular protein aggregates. These aggregates, or more likely their intermediate oligomeric precursor forms, can act to catalyze the process of additional aggregation.[9]

A highly conserved class of proteins called molecular chaperones has evolved to prevent inappropriate interactions within and between non-native polypeptides, to enhance the efficiency of *de novo* protein folding, and to promote the refolding repair of proteins that have become misfolded as a result of cellular stress.[10-11] In addition to this protein repair activity, chaperones can mediate targeting to the proteasome system or to lysosomes, resulting in selective degradation of the misfolded protein when the chaperones cannot repair the misfolded proteins.

Phenylbutyrate, an orally active chemical chaperone approved by the US Food and Drug Administration for the treatment of urea cycle disorders, mimics the function of intracellular molecular chaperones in preventing protein aggregation and oligomerization.[12]

Nogalska et al. reported a novel function of phenylbutyrate, namely that in lysosomal activity-inhibited CHMFs it substantially: (a) improved the phenotype of muscle fibers by decreasing their vacuolization; (b) increased cathepsins D and B activities, accompanied by a decrease of NBR1, p62 and LC3-II; (c) decreased

Aβ42 and Aβ42 oligomers; and (d) decreased γ-secretase activity.[13] This improvement of lysosomal activity and striking ameliorative consequences of experimentally impaired autophagy provide a rationale for considering phenylbutyrate as a potentially beneficial drug for IBM.

Study design

We conducted a pilot study (phase 1 clinical trial) to evaluate the safety and tolerability of phenylbutyrate in IBM. In this open-label study, the plan was to enroll 10 patients with sporadic inclusion body myositis who would be treated with phenylbutyrate (3 gm twice daily) for 3 months. There would be a run-in period of 3 months, during which no study medication would be taken, and certain exploratory biomarkers would be measured at baseline and at the end of the run-in period in addition to final measurement at the end of the treatment period.

These biomarkers evaluate the mitochondrial function, stress response, degenerative process and apoptosis and include mitochondrial membrane potentials through MitoTracker red (shows overall mitochondrial mass), MitoSox (a mitochondrial biomarker which detects mitochondrial superoxide levels), tetramethylrhodamine ethyl ester (determine mitochondrial membrane potential and a marker of overall mitochondrial activity), Annexin binding in lymphocytes (apoptosis marker). We have previously assayed TDP-43 and its derivative, phosphorylated TDP-43 (pTDP-43) in peripheral blood platelets in our laboratory (Dr. Agbas). TDP43 positive cytoplasmic aggregates have been described in ALS motor neurons and IBM skeletal muscle tissue with significant up-regulation of *TARDBP* (the gene expresses TDP-43 protein) and *SQTM1* (Sequestosome-1) gene expression.[14] Therefore, we planned to measure blood-derived platelet total TDP-43 and pTDP-43 as surrogate biomarker for skeletal muscle TDP-43 profile of IBM patients. Our laboratory had demonstrated analytical capability of the measurement of platelet TDP-43 and pTDP-43 in human platelets.[15,16,17]

Primary outcome measures included adverse event reporting. Secondary outcome measures included multiple strength and functional measures: manual muscle testing, timed up and go test (TUG), IBM functional rating scale (IBMFRS), and grip strength. These clinical outcome measures were commonly used in evaluating progression of IBM and in clinical trials of this disease. [18,19,20,21] In addition, safety laboratory tests and electrocardiogram would be monitored regularly. As mentioned above, the biomarkers would be repeated at the end of the treatment period.

Study Eligibility

Inclusion Criteria

1. Fulfill ENMC 2011 diagnostic criteria for IBM
2. Age ≥ 18 years
3. Women must be post-menopausal (no menses in >12 months) or status post hysterectomy.
4. Able to give informed consent
5. Subjects must be able and willing to remain on stable concomitant medications throughout the duration of the study.

Exclusion Criteria

1. Presence of any one of the following medical conditions: chronic infection; chronic renal insufficiency; cancer other than skin cancer less than five years prior; multiple sclerosis or prior episode of central nervous system demyelination; or other chronic serious medical illnesses
2. Presence of any of the following on routine blood screening: WBC<3000; Platelets < 100,000; hematocrit < 30%; BUN > 30 mg/dl; creatinine > 1.5 mg/dl; liver disease with serum albumin < 3 g/dl
3. Women who are pregnant or lactating
4. History of non-compliance with other therapies
5. Coexistence of other muscular disease
6. Drug or alcohol abuse within the past three months
7. Known bleeding disorder
8. Known liver disease
9. Known congestive heart failure
10. Known hypernatremia

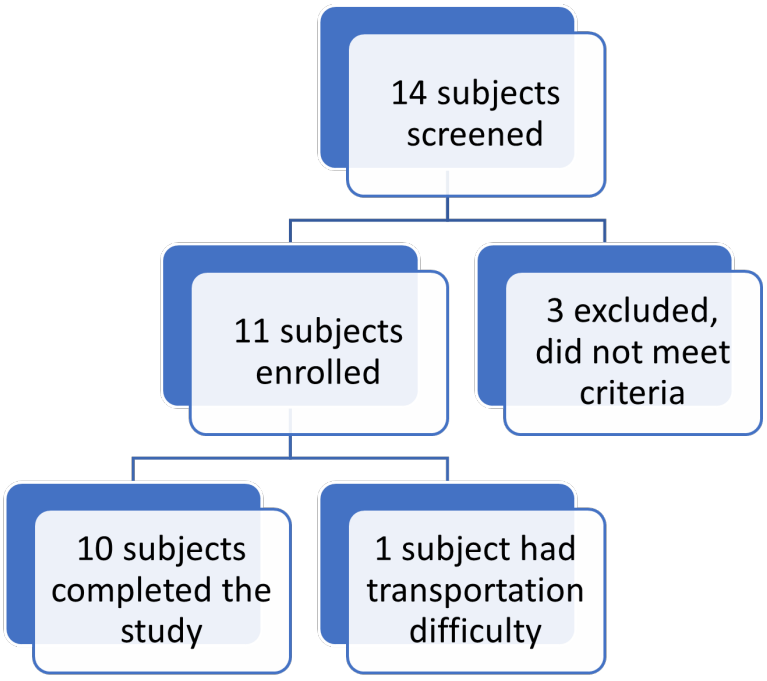
Statistical Method

This is a pilot study with a sample size of n=10 subjects. For both the primary and secondary outcomes, statistical reporting would be in the form of descriptive statistics.

Demographic variables are reported using mean and standard deviation (or median) for continuous variables (age, weight, height) and frequency counts for categorical variables (gender, race, ethnicity). The disposition to complete or not complete treatment would be recorded as a 'Yes/No' dichotomous response and would be reported using frequency counts.

For the primary outcome of safety, the frequency of Adverse Events (AEs) and Serious Adverse Events (SAEs) by grade, would be reported. This would be done for each follow-up visit as well as for all visits combined.

For the secondary outcomes, the measurements at baseline, month-3, and month-7 would be reported using mean and standard deviation (or median and interquartile range for skewed measurements) along with a corresponding 95% confidence interval. These measurements would be visually compared using box plots to detect the presence of a trend over time by drawing a trend line on the means (or median). Due to the small sample size, this comparison would be done visually, and no formal statistical test would be done routinely but it would be considered to further study a trend.



Results

Fourteen patients with IBM were screened for this study. Three patients were excluded from the study due to not meeting the inclusion/exclusion criteria. Ten patients were enrolled in the study, but one subject dropped out of the study due to transportation difficulties. After getting IRB approval, one additional subject was enrolled, and all 10 subjects completed the study.

The demographic characteristics can be seen in **Table 1 (a, b)**. F:M ratio was 7:4 and median age was 73 (67, 76).

Table 1a. Demographics Characteristics

	Participants
Enrolled, N	11
Female N (%)	7 (63.6%)
Caucasian N (%)	10 (90.9%)
Age (years median (IQR))	73 (67, 76)

Table 1b – Detailed Demographics

Subject number (enrolled only, n=11)	Gender	Age	Race
1	F	73	Caucasian
2	F	72	Caucasian
4	F	77	Caucasian
5	M	91	Caucasian
6	F	74	Caucasian
7	M	57	Caucasian
9	F	76	Hispanic or Latina
10	M	80	Caucasian
11	F	77	Caucasian
13	F	68	Caucasian
14	M	67	Caucasian

The study drug was well tolerated, and all enrolled subjects were able to continue it throughout the treatment period. There was one serious event (a fall that required a hospital visit) that was not related to the study drug. No study drug-related serious events were reported.

Two subjects had mildly elevated liver enzymes at baseline before the treatment period. No other significant laboratory changes were noted. One subject had hair loss which was considered possibly related. The most common adverse events were related to gastrointestinal symptoms (n=9), **(Tables 2 and 3)**.

The IBMFRS did not seem to change during the study (Median was 23 at baseline, 24 at the beginning of the treatment period, and 23 at the end of it), (**Figure 1)**.

There were no meaningful changes in the other clinical outcome measures either (TUG, Total MMT, Dynamometer of the knee extension or hand grips), **(Figure 2).**

Figure 1- IBMFRS Changes

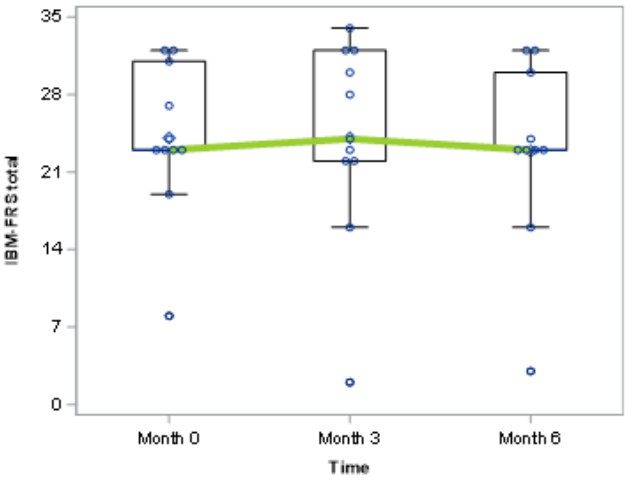


Table 2 – Adverse Events (considered possibly or probably related)

Adverse Events (possibly or probably related)		Number of occurrences	Number of subjects
Gastrointestinal AEs		9	9
	Heartburn	4	4
	Nausea	2	2
	Urgency	1	1
	Constipation	1	1
	Oral Aphthous Lesion	1	1
Musculoskeletal Pain		2	2
Drowsiness		1	1
Tremor		1	1
Hair Loss		1	1
Two Itchy Skin Lesions		1	1
Nasal Congestion		1	1

Table 3 – Adverse Events (considered unrelated)

Adverse Events (considered unrelated)	Number of occurrences	Number of subjects
Falls	9	6
Urinary Urgency	2	2
Elevated blood pressure	2	2
Elevated liver enzymes	2	2
Low blood pressure	1	1
Headache	1	1
Chills	1	1
Numbness in the Legs	1	1
Back Pain	1	1
Elevated White Blood Count	1	1
Right Bundle Branch Block	1	1
Strep. Throat Infection	1	1

Figure 2 – TUG, MMT and Dynamometer changes

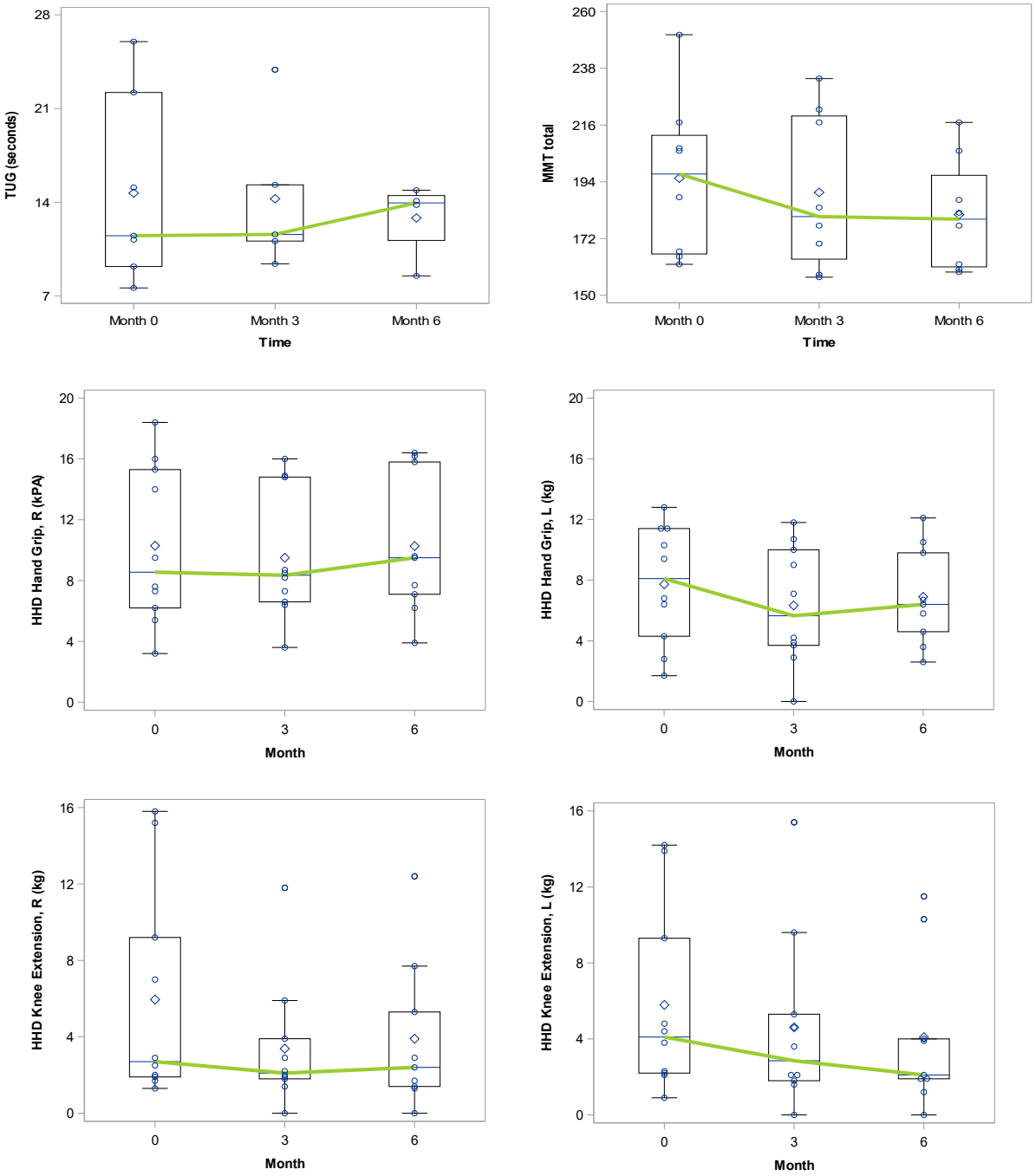
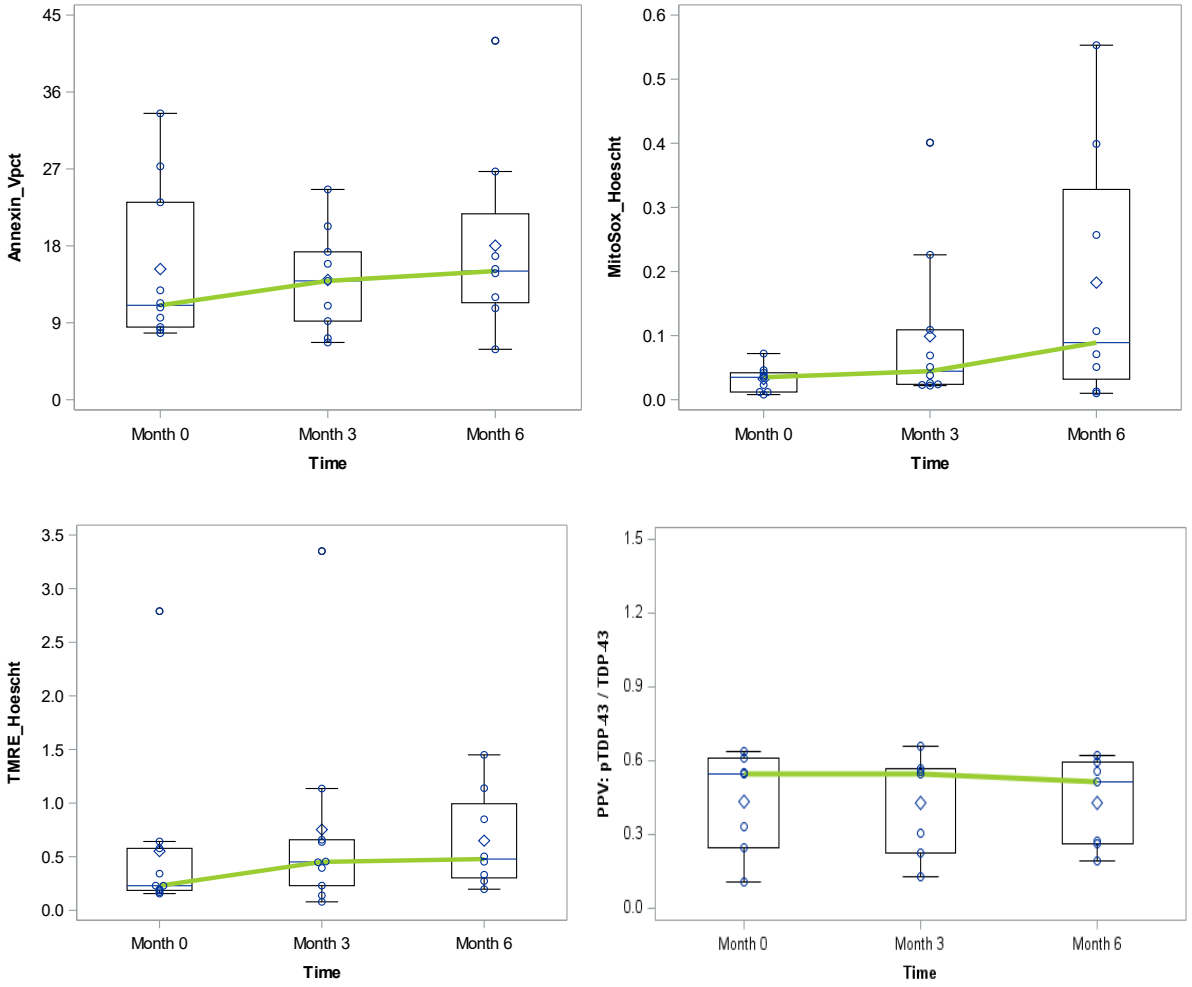
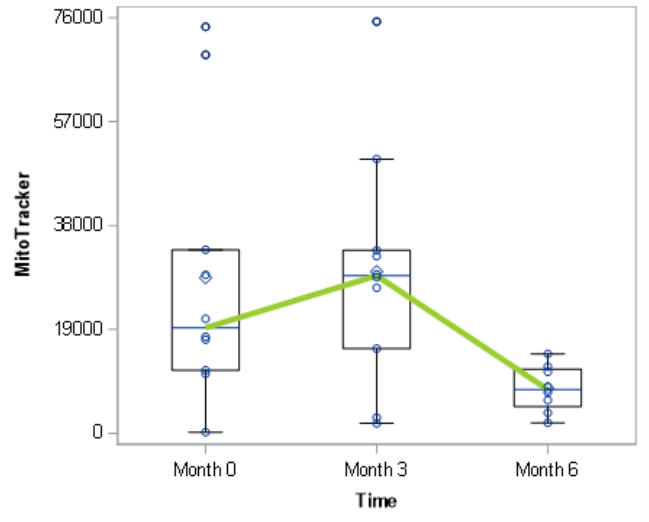


Figure 3 – Changes in Annexin, TMRE, MitoSox and TDP 43



While there was no meaningful change in most exploratory biomarkers used in this study **(Figure 3)**, the MitoTracker dropped at the end of the treatment period (median of 8010 comparing to 19299 at the baseline and 28823 at the beginning of the treatment period **(Figure 4)**. This was investigated further with a statistical analysis (detailed below in **Table 4 and Figure 5**) which showed that the mean change between the run-in period and treatment period was statistically significant (p=0.0243).

Figure 4 – Changes in MitoTracker



Statistical Analysis

Box plots of outcomes provide for visual inspection of the observed values as well as a representation of the median, inter-quartile range, and mean.

The trend observed in the boxplots of MitoTracker data warranted further exploration with a post hoc analysis. A generalized linear mixed model was used to estimate mean values of MitoTracker at each timepoint. 10 of 11 subjects had measurements at all 3 timepoints. Time was used as a categorical value and correlation among an individual's repeated measurements was accounted for with

Table 4 – statistical model

Effect	Estimated coefficient (95% CI)	Std Err	df	p-value
Intercept	29,574 (15,596, 43,552)	6,274	10	0.0008
Month			2/15	0.0724[F]
Month 0	-1,128 (-20,038, 17,782)	8,872	15	0.9005
Month 3	0	reference		
Month 6	-21,344 (-41,402, -1,287)	9,410	15	0.0385
	Mean Estimated Change (95% CI)	**Std Err**	**df**	**p-value**
On treatment – Off treatment	20,780 (3,091, 38,469)	8299	15	0.0243[t]

F: An F-test of fixed effects

t: t-test comparing mean change to zero.

a random effect for subject. The fixed effect, month, was not statistically significant (p=0.0724). Although, MitoTracker values after treatment are statistically significantly different from pre-treatment, months 0 and 3 combined (p=0.0243). The results from the model are exploratory.

Discussion

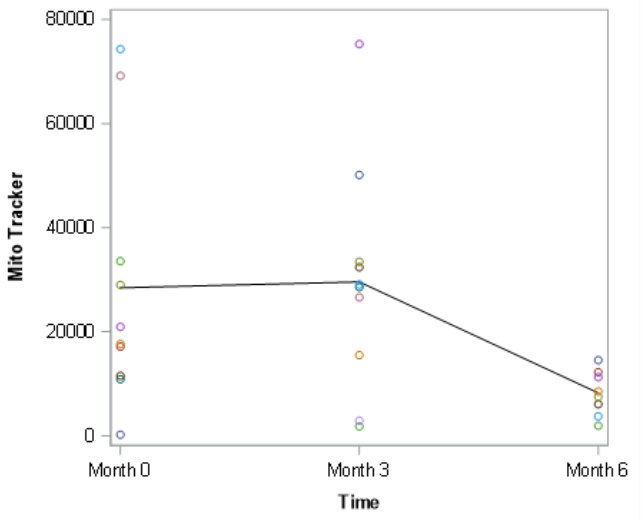
This is the first clinical trial of phenylbutyrate (PBA) in inclusion body myositis (IBM), and it was justified by the potential benefits of PBA in IBM as detailed above. This is a phase 1 trial to study the safety and tolerability of PBA in IBM.

We found PBA to be safe and well-tolerated in subjects with IBM. There were no concerning safety findings in this study and the only serious event reported (a fall) was related to the disease itself, not the study drug.

There were no meaningful changes in all clinical and most biological outcome measures. This was not unexpected considering the short duration of this study and the small sample size. Despite these limitations, the MitoTracker showed a significant drop with treatment. Reduced MitoTracker with treatment could be due to reduced mitochondrial number/mass. We propose that PBA induces autophagy/mitophagy as it is known to reduce protein aggregation and one way to process aggregates is to ship them to mitochondria and then induce mitophagy.

A phase 2 study is needed to verify the MitoTracker change and to further evaluate for any clinical or biological outcome measures changes that might have been missed due to the small sample size and short duration of our pilot study. Studying the muscle tissue should be considered in any future trial to evaluate for histopathological changes

Figure 5 – Mean Change of MitoTracker



secondary to treatment with PBA considering the changes found in the muscle cell model of IBM when exposed to PBA.[13]

In addition, PBA with tauroursodeoxycholic acid (TUDCA) showed positive results in a phase 2 clinical trial in another neurodegenerative disorder, amyotrophic lateral sclerosis (ALS). This further supports the need for a larger and longer study to evaluate its efficacy in IBM, which likely shares pathophysiological mechanisms with ALS.

Conclusion

Phenylbutyrate was safe and well tolerated in patients with IBM in this small pilot study. The change in the

MitoTracker suggests target engagement, but a longer and larger study is needed to confirm that and study the efficacy of PBA in IBM. Such Phase II study may include tissue markers to further evaluate PBA effect on the treated muscles. If PBA is found to be beneficial for the treatment of IBM, it would represent the first effective treatment for this progressive and debilitating disease.

Acknowledgement

We would like to acknowledge and thank Dr. Valerie Askanas for sharing her research findings on phenylbutyrate with us which inspired this pilot study of inclusion body myositis.

We would also like to thank Sigmapharm Laboratories for providing the study drug which made it possible to translate this research idea to a clinical trial.

References

1. Felice KJ, North WA. Inclusion body myositis in Connecticut: observation in 35 patients during an 8-year period. Medicine (Baltimore) 2001:80:320-7

2. Wilson FC, Ytterberg SR, St Sauver JL, Reed AM. Epidemiology of sporadic inclusion body myositis and polymyositis and Olmsted County, Minnesota. J Rheumatol. 2008:35:445-7

3. Dimachkie MM, Barohn RJ. Inclusion body myositis. Curr Neurol Neurosci Rep. 2013;13:321

4. Machado PM, Dimachkie MM, Barohn RJ. Sporadic inclusion body myositis: new insights and potential therapy. Curr Opin Neurol. 2014 Oct;27(5):591-8.

5. Askanas V, Engel WK. Inclusion-body myositis: a myodegenerative conformational disorder associated with Abeta, protein misfolding, and proteasome inhibition. Neurology 2006;66(2 Suppl 1):S39-48

6. Nogalska A, D'Agostino C, Engel WK, Klein WL, Askanas V. Novel demonstration of amyloid-β oligomers in sporadic inclusion-body myositis muscle fibers. Acta Neuropathol 2010;120(5):661-6

7. Nogalska, A., D'Agostino, C., Terracciano, C., Engel, W.K., Askanas, V., 2010b. Impaired autophagy in sporadic inclusion-body myositis and in endoplasmic reticulum stress-provoked cultured human muscle fibers. Am. J. Pathol 2010;177:1377–1387

8. Nogalska A, D'Agostino C, Engel WK, Askanas V. Activation of the γ-secretase complex and presence of γ-secretase-activating protein may contribute to Aβ42 production in sporadic inclusion-body myositis muscle fibers. Neurobiol Dis. 2012;48(1):141-9

9. Forloni G, Terreni L, Bertani I et al. Protein misfolding in Alzheimer's and Parkinson's disease: genetics and molecular mechanisms. Neurobiol Aging. 2002;23:957-976

10. Hartl F, Hayer-Hartl M. Molecular chaperones in the cytosol: from nacent chain to folded protein. Science. 2002;295:1852-1858

11. Sherman MY, Goldberg AL. Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. Neuron 2001; 29(1): 15-32.

12. Papp E, Csermely P. Chemical chaperones: Mechanisms of action and potential use. Handb Exp Pharmacol 2006;172:405-16

13. Nogalska A, D'Agostino C, Engel K.W, Askanas V. Sodium phenylbutyrate reverses lysosomal dysfunction and decreases amyloid-β42 in an in vitro model of inclusion-body myositis, Neurobiol. Dis 2014;65:93-101

14. Cykowski MD, Powell SZ, Appel JW et al. Phosphorylated TDP-43 (pTDP-43) aggregates in the axial skeletal muscle of patients with sporadic and familial amyotrophic lateral sclerosis. Acta Neuropathol Commun. 2018;6(1):28

15. Statland JM, Moore D, Wang Y et al. Rasagaline for amyotrophic lateral sclerosis: A randomized, controlled trial. Muscle Nerve. 2019;59:201-207

16. Wilhite R, Sage JM, Bouzid A, Primavera T, Agbas A. Platelet phosphorylated TDP-43: an exploratory study for a peripheral surrogate biomarker development for Alzheimer's disease. Future Sci OA. 2017;3:FSO238

17. Sage JM, Hall L, McVey A, Barohn RJ et al. Use of capillary electrophoresis immunoassay to search for potential biomarkers of amyotrophic lateral sclerosis in human platelets. J Vis Exp. 2020:156

18. Jackson CE, Barohn RJ, Gronseth G, Pandya S, Herbelin L; Muscle Study Group. Inclusion body myositis functional rating scale: a reliable and valid measure of disease severity. Muscle Nerve. 2008;37:473-6

19. Jørgensen AN, Aagaard P, Nielsen JL et al. Physical function and muscle strength in sporadic inclusion body myositis. Muscle Nerve. 2017;56:E50-E58.

20. Lin AY, Siener CS, Faino AV et al. Optimizing hand-function patient outcome measures for inclusion body myositis. Neuromuscul Disord. 2020;30;807-814

21. Sangha G, Yao B, Lunn D et al. Longitudinal observational study investigating outcome measures for clinical trials in inclusion body myositis. J Neurol Neurosurg Psychiatry. 2021;jnnp-2020-325141