Can the Therapeutic Efficacy of Tocotrienols in Neurodegenerative Familial Dysautonomia Patients Be Measured Clinically?

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ABSTRACT

Familial dysautonomia (FD) is an inherited, fatal, neurodegenerative disorder manifested by autonomic/hypertensive crises and cardiac instability. Patients produce little IKAP, the gene product of the affected mutated gene, and have low levels of monoamine oxidase A (MAO A), whose reduced presence appears to result in an increased accumulation of biogenic amines, which is a trigger for hypertensive crises. As ingestion of tocotrienols elevates IKAP and MAO A in FD patients, we examined their impact on the frequency of hypertensive crises and cardiac function. After 3 to 4 months of tocotrienol ingestion, 80% of patients reported a significant (≥50%) decrease in the number of crises. In a smaller group of patients, a postexercise increase in heart rate and a decrease in the QT interval were observed in the majority of participants. Based on these findings, we hypothesize that tocotrienol therapy will improve the long-term clinical outlook and survival of individuals with FD.

INTRODUCTION

Familial dysautonomia (FD), also known as Riley–Day syndrome, is an autosomal recessive disorder that affects the development and survival of sensory and sympathetic neurons and occurs primarily among those of Ashkenazi Jewish descent (11, 13). Individuals with FD manifest a variety of symptoms, including cardiac instability, misdirected swallowing, delayed developmental milestones, unsteady gait, poor balance, decreased perception of pain and temperature, an absence of overflow tears, and autonomic/hypertensive “crises.” These crises are characterized by protracted periods of nausea and vomiting accompanied by hypertension and tachycardia (5).

Clinical management thus far has consisted primarily of attempting to control dysautonomic symptoms, mainly with adrenergic agonists, fludrocortisone, and benzodiazepines (5). Analysis of the sensory responsiveness of individuals with FD reveals that FD is a progressive neurologic disorder leading to an increased loss of neuronal function over time (10). FD is a life-threatening disorder with a high mortality rate. Death is often the result of long-term cardiorespiratory complications or strokes that occur as a result of the hypertensive crises. A study of two populations of individuals with FD diagnosed at or below the age of 2 years has revealed that they have a cumulative death rate of 31% and 19%, respectively, within the subsequent 10 years (9).

Mutations in the IKBKAP gene, which encodes the IκB kinase complex–associated protein (IKAP), cause FD (1, 24). IKAP was originally reported to be a scaffold protein involved in the assembly of the IκB kinase complex (14), but subsequent studies reveal that it is not involved in IκB kinase complex assembly (19) and is likely a component of the Elongator complex (17, 20) or is a c-Jun N-terminal kinase (JNK)-associated protein (18) or both. The role IKAP plays in the development and maintenance of neurons is unknown and studies are currently under way to determine why low levels of IKAP result in FD. The most common, or major, FD-causing mutation is a T→C transition in the sixth base of the donor splice site of in-
tron 20, which changes the donor splice-site sequence from the consensus GTAAGT to GAAAGC and results in the generation of a misspliced transcript that lacks exon 20. Translation of this mRNA results in a frameshift and produces a truncated protein lacking all of the amino acids encoded by exons 20–37 of the IKBKAP gene. Study of the IKAP transcript in cells and tissues of FD patients revealed that the IVS20+61G→C mutation is somewhat leaky, allowing some correctly spliced RNA and, therefore, some full-length protein to be generated (2, 15). In light of this leakiness, we evaluated the impact of various agents on the level of full-length (wild-type) IKAP transcript produced in FD-derived cells. We demonstrated that tocotrienols, by increasing transcription of the IKBKAP gene, elevate the level of the wild-type transcript (2). In addition, epigallocatechin gallate (EGCG), a flavonoid found in green tea, corrects the aberrant splicing of IKAP and elevates the levels of the wild-type IKAP transcript and protein in FD-derived cells (3).

The hypertensive crises occurring in individuals with FD are often triggered by emotional or physical stress that results in a marked elevation of plasma norepinephrine (NE) and dopamine (DA) levels (5). Examination of levels of monoamine oxidases (MAO) A and B, key enzymes involved in the degradation of biogenic and dietary amines, in FD-derived tissues revealed a reduced presence of MAO A (4). Tocotrienols and EGCG treatment of FD-derived cells and the ingestion of tocotrienols by individuals with FD results in a concomitant elevation of IKAP and MAO A levels (4). The observed tocotrienol-mediated elevation of MAO A levels in individuals with FD prompted an analysis of the impact of tocotrienol ingestion on the frequency of dysautonomic crises in this patient population.

In addition, in light of the compromised cardiac function observed in individuals with FD (16) and the role cardiorespiratory complications play in the high morbidity/mortality of FD (12), the impact of tocotrienol ingestion on the postexercise cardiac response of individuals with FD was studied.

**CLINICAL EFFECTIVENESS OF TOCOTRIENOLS IN FD PATIENTS**

The observed ability of tocotrienols to (a) increase functional IKAP mRNA and protein production in FD-derived cells (2); and (b) increase functional IKAP and MAO A mRNA levels in peripheral blood cells of individuals with FD (4), prompted a clinical investigation of the impact of tocotrienol ingestion on individuals with FD. Although a double-blind, randomized, comparative trial would most conclusively investigate the clinical impact of tocotrienol ingestion on individuals with FD, for us, the possibility of using tocotrienols, which have been shown to have no adverse side effects at doses as high as 200 mg/day (21, 22) and have been suggested to be safe for human consumption at up to 1,000 mg/day (25), to mitigate the progressive neuronal degeneration and high morbidity/mortality observed in individuals with FD, posed an ethical dilemma that prompted us to perform a prospective, open label, noncomparative case series.

By using a questionnaire that examined the frequency of hypertensive crises before and after a period of tocotrienol ingestion, we interviewed the parents of 26 individuals who experienced hypertensive crises, asking them to report on the frequency of crises before starting tocotrienol supplementation and then after the patient had been taking 100 mg of TwinLab MaxiLIFE Tocotrienols per day for 3–4 months. Of the 26 that were initially enrolled in the study, 23 reported taking the tocotrienols daily, and their responses were evaluated: 82.6% of these participants reported a reduction in the frequency of crises, and 39.1% reported a complete cessation of crisis activity (Table 1). The remainder saw neither a decrease nor an increase in the frequency of hypertensive crises.

Glickstein and co-workers (16), in 1993, reported that individuals with FD fail to exhibit an increased pulse rate or a shortening of the QTc interval after exercise (16). In light of the role compromised cardiac function plays in the high morbidity/mortality of FD patients, we examined the impact of tocotrienol ingestion on cardiac function. For this study, we recruited five patients ranging in age from 6 to 34 years. Before and 3–4 months after initiating daily ingestion of 100 mg of tocotrienol, we examined the resting/baseline pulse rate and QT intervals of the participants and then measured the impact of exercise (20 knee bends) on their cardiac response. Before initiating tocotrienol ingestion, all five of the enrolled participants exhibited either no change or a decrease in their pulse rate, and most had only a very modest shortening of the QTc interval in response to exercise. After a period of 3 to 4 months of tocotrienol supplementation, all of the participants exhibited an exercise-mediated increase in pulse rate, and three of the four evaluable participants showed a shortening of the QTc interval (Fig. 1). We could not evaluate QTc intervals for one patient because of the lack of discernable T waves in the standard II derivation.

**CONCLUSIONS AND OPEN QUESTIONS**

FD is an autosomal recessive disorder that results in the dysfunction of the autonomic and sensory nervous systems. A primary manifestation of FD is the autonomic crisis, which can be a life-threatening event that can result in stroke and death. Although the life expectancy of those with FD has increased in recent years, many children die before their teenage years, and very few survive into their 30s or beyond (9). The progressive neurologic loss of function that occurs over time generates increasing challenges to the older FD patient (10). Therapeutic modalities used to date are designed to control the symptoms manifested by FD but do not address the underlying deficiency of IKAP that causes this disease (6).

**Table 1. Impact of Tocotrienols on Hypertensive Crises in FD Patients**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number (percentage of total)</th>
</tr>
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<tbody>
<tr>
<td>No impact</td>
<td>4 (17.4%)</td>
</tr>
<tr>
<td>Fewer crises (≥50% reduction)</td>
<td>19* (82.6%)</td>
</tr>
<tr>
<td>Complete cessation of crises</td>
<td>9* (39.1%)</td>
</tr>
</tbody>
</table>

*Patients with “complete cessation of crisis” were included in the “fewer crises” group.
The leaky nature of the major FD-causing mutation prompted an investigation of the ability of substances to increase the amount of the functional transcript produced by the mutated \textit{IKBKAP} gene. The ability of tocotrienols to increase transcription of \textit{IKBKAP}, and because of the leakiness of the IVS20\textsuperscript{+6T\rightarrow}C mutation, generate more functional gene product in cell lines derived from FD patients and in FD patients, led us to evaluate the impact of tocotrienol supplementation on the symptoms manifested in individuals with FD. In this study, we demonstrated for the first time that ingestion of tocotrienols by individuals with FD results in reduced frequency of dysautonomic crises and an enhanced cardiac responsiveness to exercise, thereby definitively demonstrating that ingestion of tocotrienols results in a clinically measurable outcome. Although somewhat more difficult to measure, the results of the questionnaire surveys also provided evidence that tocotrienol ingestion by those with FD resulted in an increase in eye moisture (data not shown) and, for some patients, the elimination of the need for daily use of eye drops.

As the tocotrienol preparation used in this study also contained tocopherol, we cannot eliminate the possibility that the tocopherols are playing a role in the observed biologic response. However, because the tocopherols fail to modulate \textit{IKBKAP} gene expression \textit{in vitro} (2), and many of the participants who had been taking a tocopherol supplement before enrolling in this study exhibited a positive response to the tocotrienol supplementation, it is unlikely that the tocopherol present in the TwinLab tocotrienol supplement is mediating the observed effects. It remains to be elucidated how the tocotrienols increase transcription of \textit{IKBKAP} and why the tocopherols fail to mediate this effect.

Survival statistics reveal that many of those with FD die as a result of complications of hypertensive crises or of cardiorespiratory arrest. It has been suggested that the abnormal cardiac function in patients with FD plays a role in these deaths (7). It is thus likely that the observed tocotrienol-mediated reduction in the number of hypertensive crises and the improvement in cardiac function will provide a survival benefit for those with FD. Clinical monitoring of survival in the patient population taking tocotrienols has already suggested a reduced mortality compared with those not taking the tocotrienols.

\textbf{FIG. 1. Heart rate and ECG responses to exercise in five individuals with FD.} \textit{Top:} Average change from the resting heart rate in the first 3 min after 20 knee bends. Resting heart rate was determined as the average heart rate across 10 min before exercise. \textit{Open bars,} Heart-rate responses before tocotrienol supplementation. \textit{Solid bars,} Heart-rate responses 3–4 months after the initiation of tocotrienol supplementation. \textit{Bottom:} Changes in the duration of QT\textsubscript{c} intervals of the ECG in response to 20 knee bends. QT\textsubscript{c} intervals were averaged for the last three cardiac cycles before exercise and for the first three cardiac cycles after exercise. The differences between post- and preexercise averages are shown before tocotrienol supplementation (\textit{open bars}) and 3–4 months after the initiation of tocotrienol supplementation (\textit{solid bars}). T waves could not be detected in patient 1 in standard II ECG derivation, and therefore the data were not available (N/A). Error bars represent standard error.
Tocotrienols possess potent neuroprotective, antioxidant, anticancer, and cholesterol-reducing properties (23). The therapeutic use of tocotrienols for individuals with FD represents the first direct approach that addresses the genetic deficiency that causes FD. Additional clinical studies will be required to determine the optimal dosage and the frequency of tocotrienol administration. Although the ingestion of tocotrienols results in an elevation of IKAP levels and, as a result, mitigation of the impact of the FD-causing mutation present in the IKAP-encoding gene, the mechanism by which IKAP affects neurologic function remains to be determined. In FD, a progressive loss of neurologic function is due to a loss of neurons over time. Does the increase in IKAP levels halt or even reverse some of the neuronal loss, leading to the symptomatic improvements that have been observed? An understanding of the IKAP biologic activities, in general, and in the tocotrienol-induced clinical improvements seen in FD patients, may provide guidance regarding other medical conditions that would benefit from the tocotrienol-mediated elevation of IKAP levels.

APPENDIX

Notes

1. Patient population
Individuals enrolled, with informed consent and approval from the institutional review board of Fordham University, were all homozygous for the IVS20+6T–C mutation.

2. Tocotrienol supplementation
Individuals participating in this study took oral doses of 100 mg per day (two capsules) of MaxiLIFE Rice Tocotrienols (TwinLab, Hauppauge, NY). Each capsule contains 47 mg of gamma tocotrienol, 2 mg of alpha tocotrienol, 1 mg of delta tocotrienol, and 50 I.U. of α-alpha tocopherol.

3. Questionnaire data collection
Twenty-six individuals with FD, ranging in age from 4 to 34 years, were solicited to enroll in this study. Parents were interviewed either by phone or in person at various times during the study and asked to provide information on the number of hypertensive crises experienced by phone or in person at various times during the study and asked to provide information on the number of hypertensive crises experienced before and after initiating supplementation with the tocotrienols. Participants were considered nonevaluable if they stopped taking the supplement during the study. The responses of 23 individuals were evaluable.

4. Measurement of cardiac function
Standard bipolar II ECG was recorded by a computerized system (MP100 System; Biopac Systems Inc., Goleta, CA) before starting the tocotrienol treatment and 3–4 months after the start of the treatment. During each recording session, two 5-min baseline segments were recorded, the first in supine, and the second in standing position; the ECG was recorded for an additional 5 min. Average heart rates were measured across the 10-min baseline and across the first 3 min of the postexercise periods. In addition, QT intervals were measured and corrected for the length of the cardiac cycle as follows: QTc, measured QT interval (msec)/square root of preceding RR interval (sec). QTc values were calculated and averaged for the last three consecutive cardiac cycles of the baseline periods and for the first three consecutive cardiac cycles after the end of exercise. QTc intervals could not be calculated for one of the subjects because of the lack of discernable T waves in the standard II derivation.

ABBREVIATIONS

EGCG, epigallocatechin gallate; FD, familial dysautonomia; IKAP, IκB kinase complex–associated protein; JNK, c-Jun N-terminal kinase; MAO, monoamine oxidase; QTc, QT correction; RT-PCR, reverse transcriptase polymerase chain reaction.

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