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Platelet function in stroke/transient ischemic attack patients treated with tocotrienol

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Abstract

The purpose of this study was to characterize the effects of tocotrienol form of vitamin E (TCT) on platelet function in patients with stroke or transient ischemic attack (TIA). A double blind, randomized, single center phase II clinical trial was conducted comparing placebo (PBO) and 400 and 800 mg TCT daily for a year in 150 patients with a sentinel ischemic stroke or TIA event in the prior 6 months. Platelet function was measured at baseline and then, at 3 month intervals for a year, using light transmission aggregometry. The incidence of aspirin resistance in aspirin-treated patients or platelet inhibition in patients on clopidogrel alone was compared between the three treatment groups. Results showed that in patients taking aspirin and clopidogrel, the incidence of aspirin resistance was significantly decreased from 40% in PBO-treated patients to 9% in the 400 mg TCT group and 25% in the TCT 800 mg group ($P = .03$). In conclusion, patients on aspirin and clopidogrel had a higher incidence of aspirin resistance than all patients treated with aspirin alone and TCT decreased the frequency of aspirin resistance in this group.

KEYWORDS

aspirin resistance, clinical trial, nutrition, vitamin E

1 | INTRODUCTION

Aspirin decreases the relative risk of recurrent stroke, myocardial infarction, and vascular death by 13%, and nonfatal ischemic stroke by 19% in patients who have had a transient ischemic attack (TIA) or stroke.[1] While other antiplatelet agents such as clopidogrel and extended release dipyridamole plus aspirin may be modestly more effective than aspirin alone in preventing stroke or combined cardiovascular endpoints,[2] other medications are needed to approach the 62% relative reduction of stroke risk with dose-adjusted warfarin in patients with atrial fibrillation.[3] Some of the recurrent strokes seen in patients on aspirin and clopidogrel may relate to the failure of these agents to inhibit platelet aggregation in vitro.[4] However, these effects are dependent on the platelet function test used, may be dose-dependent, and the importance of these tests in predicting increased risk of cardiovascular events is unclear and requires further study.[5,6]

Vitamin E is a generic term for tocopherols (TOC) and tocotrienols (TCT). TCT have functions in health and

Abbreviations: IRB, Institutional Review Board; LTA, light transmission aggregometry; TCT, tocotrienol; TIA, transient ischemic attack.

Andrew Slivka and Cameron Rink contributed equally to this work.

disease that are clearly distinct from that of TOC.[7,8] In preclinical studies, TCT have been shown to inhibit platelet thrombus formation and aggregation in stenosed canine coronary arteries.[9] A pilot study in normal volunteers suggested TCT has antiplatelet effects similar to aspirin in about 50% of patients (Table 1), though no dose response was seen possibly due to a ceiling effect. Furthermore, micromolar amounts of TCT, not TOC, suppressed the activity of hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase).[10,11] HMG-CoA reductase is the same enzyme targeted by the statin class of drugs that have been found to be beneficial in decreasing the risk of recurrent stroke.[12] The NUTRITION Trial was designed to characterize the effects of TCT on platelet function, lipids, and safety in stroke patients receiving the standard of care treatment for secondary stroke prevention. Here, we report the platelet function results. We hypothesized that TCT would decrease the incidence of aspirin resistance by 10% in patients taking aspirin or aspirin and clopidogrel based on the pilot results that platelet inhibition using arachidonic acid would be seen in 50% patients on clopidogrel alone.

2 | SUBJECTS/MATERIALS AND METHODS

2.1 | Clinical studies

All procedures performed in studies involving human participants (clinicaltrials.gov NCT01858311) were in accordance with the ethical standards of the institutional and/or national research committee (The Ohio State University Institutional Review Board (IRB#2011H0242) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.2 | Informed consent

Informed consent was obtained from all individual participants included in the study.

The NUTRITION trail was designed as a single center, randomized double blind phase II trial. Patients with atherothrombotic, cardio-embolic, cryptogenic TIA or stroke within 6 months of clinical presentation for whom anticoagulation was not indicated, with a post stroke modified Rankin Score (mRS) < 4, were assigned to placebo, 400mg TCT, or 800 mg TCT daily. Exclusion criteria included prior intracranial hemorrhage (excluding traumatic), high risk of bleeding (recurrent gastrointestinal, genitourinary bleeding, active peptic ulcer disease), anticipated requirement for long-term use of anticoagulation, contraindications to antiplatelet agents (bleeding disorder, thrombocytopenia, prolonged INR), irreversible medical condition such as cancer or other chronic disease with predicted survival of less than a year, severe psychiatric or neurologic disease that might complicate evaluation of study outcomes like dementia or schizophrenia, pregnancy, or women of child bearing age who are not following an effective method of contraception, breast feeding, unable or unwilling to provide informed, unlikely to be compliant with therapy or unwilling to return for follow-up frequent clinic visits, concurrent participation in another study with an investigational drug or devise, other likely specific cause of stroke such as dissection, infectious or noninfectious vasculitis, prothrombotic state, no history of long-term vitamin E supplement (defined as daily oral tocopherol or TCT supplementation greater than 6 months within the past 5 years) and no current vitamin E supplementation in multivitamin.

TABLE 1 Platelet inhibition by arachidonic acid in normal volunteers taking aspirin or tocotrienol

	Number with platelet inhibition at 3 months (%)
81 mg Aspirin, n = 5	5 (100%)
400 mg TCT, n = 8	4 (50%)
800 mg TCT, n = 11	5 (45%)

Platelet function studies were performed at baseline and 3 month intervals for 1 year. Platelet aggregation was assessed in platelet rich plasma (PRP) at 37°C by light transmission aggregometry (LTA). PRP was obtained by centrifugation of citrated whole blood for 10 minutes at 1000 rpm and adjusted to 250-450 $\times$ 10^9/L with platelet poor plasma, obtained by centrifugation of the remaining blood for 10 minutes at room temperature at 3000 rpm. Aggregation was measured with a Chronolog Aggregometer (540 model, PA, USA) within 90 minutes of blood collection in all patients and was expressed as the maximal percent change in light transmittance from baseline after the addition of arachidonic acid (1.6 mM), using platelet poor plasma as a reference. Residual platelet aggregation >19% on aspirin therapy was considered aspirin resistant.[13] Compliance was measured by pill counts at each follow-up visit and patients were considered compliant if they took more than 80% of the study medication for the prior 3 months.

The outcome studied was the incidence of aspirin resistance in patients taking aspirin or aspirin and clopidogrel and incidence of platelet inhibition to arachidonic acid in patients taking clopidogrel alone. Aspirin resistance was defined by residual platelet aggregation >19% with arachidonic acid using LTA. At the start of the study the presumption was made that if a patient was aspirin resistant they would remain aspirin resistant throughout the course of the study, or at least 2-3 of the four follow-up measures. This turned out not to be the case, so the percent of the total number of follow-up visit

platelet aggregation results that were aspirin resistant were compared between each of the treatment groups who were being treated with aspirin or aspirin and clopidogrel using the Chi-square test. In the patients treated with clopidogrel alone, the incidence of platelet inhibition to <20% with arachidonic acid, for all the follow-up platelet aggregation results, was compared between the three treatment groups with the Chi-square test.

3 | RESULTS

From 3/2013 to 10/2015, 150 patient were recruited in this study (n = 49 PBO, n = 51 400 mg TCT, n = 50 800 mg TCT). There were more women, and more patients with TIA rather than stroke in the PBO group compared to the TCT treatment groups, but otherwise baseline characteristics did not differ among the three groups (Table 2). One hundred and twelve patients completed all four follow-up laboratory visits, six patients completed three of the four follow-up visits, seven completed two of the follow-ups, none patients one of the follow-up visits, and 16 completed none of the follow-up visits. Reasons for missed visits included development of conditions for which long-term anticoagulation was indicated, patient withdrawal from study or failure to respond to calls to schedule follow-up visits. Medication compliance was 64% in the PBO group, 91% in the 400 mg TCT group and 80% in the 800 mg TCT group ($P < .01$, chi-square).

At the baseline visit, 2 of 83 (2%) patients on aspirin and 1 of 33 (3%) patients on aspirin and clopidogrel were aspirin resistant. A total of (80) patients taking aspirin or aspirin and clopidogrel had platelet function testing at baseline and all four follow-up visits. Only one patient (1%) had resistance documented at all five visits and (58) patients (72%) were not resistant at any visit. Thirteen patients (16%) had resistance on one visit, six patients (8%) on two visits, two patients on three visits (3%). Of all the follow-up visits for which platelet aggregation studies were done in patients on aspirin alone, 9% were aspirin resistant in the PBO group. TCT either at 400 or 800 mg dose had no effect on the incidence of aspirin resistance in patients treated with aspirin alone (Table 3). Since as mentioned above the incidence of aspirin resistance

TABLE 2 Participant demographics

		Placebo (n = 49)	400 mg TCT, (n = 51)	800 mg TCT, (n = 50)	*P* value
Sex (M)		18	32	34	$P = .04$
Age, mean (range)		60 (33-87)	61 (35-81)	63 (32-84)	
Ethnicity	European	43	40	45	
	African American	6	10	4	
	Asian American			1	
Qualifying event	TIA	3	13	11	$P = .03$
	Stroke	46	38	39	
Etiology	Large artery atherosclerosis	9	12	15	
	Small vessel occlusion	25	22	20	
	Unknown	14	17	14	
	Cardio-embolic	1			
	Multiple			1	
mRS at entry	0, 1	34	36	35	
	2,3	15	15	15	
Risk factors	Hypertension	34	38	42	
	Hyperlipidemia	34	38	31	
	Diabetes mellitus	18	22	22	
	Smoke	10	9	6	
	Coronary artery disease	11	16	6	
	Peripheral vascular disease	5	2	0	
	prior TIA/stroke	9	9	12	

TABLE 3 Aspirin resistance in patients on aspirin and platelet inhibition in patients on clopidogrel

Antiplatelet therapy	Treatment group	Number follow-up visits resistant/Total number of follow-up visits (%)	*P* value
Aspirin	Placebo	9/99 (9%)	$P = .5$
	400 mg TCT	10/106 (9%)	
	800 mg TCT	10/115 (9%)	
Aspirin & clopidogrel	Placebo	12/30 (40%)	$P = .03$
	400 mg TCT	2/22 (9%)	
	800 mg TCT	5/20 (25%)	
		Number follow-up visits inhibited/Total number of follow-up visits (%)	
Clopidogrel	Placebo	6/26 (23%)	$P = .9$
	400 mg TCT	7/30 (23%)	
	800 mg TCT	8/41 (20%)	

on repeated visits in a single patient was low, the fact that the incidence of aspirin resistance at each of the follow-up visits was similar in all three groups (Table 4), suggests that for the most part different patients were aspirin resistant at each of the follow-up visits. There was also no difference in the aspirin responders in patients taking clopidogrel alone between the three groups (Table 3). In those taking aspirin and clopidogrel, we observed a much higher rate of 40% aspirin resistance in the PBO group than in any of the three treatment groups of patients taking aspirin alone and a significant reduction in aspirin resistance ($P = .04$) in patients on 400 mg TCT and 800 mg (Table 3). In all of the treatment groups in patients taking aspirin or aspirin and clopidogrel the incidence of aspirin resistance was lower at baseline than during the follow-up visits, though frequency remained consistent for each of the follow-up visits (Table 4).

4 | DISCUSSION

The incidence of aspirin resistance at baseline in our study population (2.5%), was lower than the range reported by others, 14%-43%, in stroke patients[14-18] though most of these patients were tested acutely using different methods of measuring platelet aggregation, different definitions of aspirin resistance and in two studies the incidence was reported after receiving an aspirin dose so there were no potential issues with compliance. The timing of the testing may also play a role in the incidence of aspirin resistance. In patients with stable coronary artery disease 4%-13% were found to be aspirin resistant,[13,17,19,20] while 53% were aspirin resistant in patients with acute coronary syndromes[21] although again different methods of measuring platelet aggregation were used in these studies. Furthermore in two studies aspirin was subsequently given to resistant patients at baseline with at least a 50% reduction in aspirin resistance suggesting that in many patients aspirin resistance is related to noncompliance.[18,20] Dose may also play a role, Gengo reported on 100 patients that were aspirin nonresponsive to 81 mg of aspirin. About 79% were responsive with 162 mg or higher. Only 6% were not responsive to any increased dose.[22]

One of the most interesting findings of this study relates to the natural history of aspirin resistance. Data on aspirin resistance over time is limited. Stejsakl reported on 103 patients with acute coronary syndrome tested within 7-9 days, then at 3, 12, 36, and 48 months.[21] There was no change in

TABLE 4 Aspirin resistance across visits in patients on aspirin and clopidogrel or aspirin alone

		Number of aspirin resistant patients at each visit			
	Baseline	**3 months**	**6 month**	**9 months**	**12 months**
Aspirin and clopidogrel					
Placebo	1/11 (9%)	3/9 (33%)	2/7 (29%)	3/7 (43%)	4/7 (57%)
400 mg TCT	0/11	0/9	1/4 (25%)	1/5 (20%)	0/4
800 mg TCT	0/11	0/8	1/3 (33%)	3/5 (60%)	1/4(25%)
Aspirin					
Placebo	1/27(4%)	2/25(8%)	3/25(12%)	2/24(8%)	2/25(8%)
400 mg TCT	0/29	3/27(11%)	1/27(4%)	3/27(11%)	3/25(12%)
800 mg TCT	1/27 (4%)	2/28(9%)	2/31(6%)	2/28 (7%)	4/28(14%)

the responders and nonresponders during that time. Nine patients that initially responded were resistant at 48 months and eight patients initially resistant were responders at 48 months. Gengo followed 86 TIA or stroke patients for an average of 196 days ± 162 days. At baseline 73 patients were responders using impedance aggregometry, and 13 nonresponders. All responders remained responders at follow-up and only 2 of 13 nonresponders changed to responders.[23] Our results are similar in that most aspirin responders remain responsive during follow-up but aspirin resistance is infrequent over time and long-term aspirin resistance is uncommon. The inconsistency of aspirin resistance over time suggests that most of the variability over time may relate to compliance rather than "true" aspirin resistance, since most patients do not remain aspirin resistant on repeated measures. Compliance may also be a potential reason for why the baseline frequency of aspirin resistance in all treatment groups on both aspirin and aspirin and clopidogrel were lower than the incidence seen in the follow-up visits for all these groups. Arguing against a compliance mechanism however, is the consistency of the lower rates in baseline visits and the higher rates in follow-up visits for all patients in the study. The explanation for this finding is, therefore, not entirely clear. Several clinical and laboratory factors have been reported to be associated with aspirin resistance including lower HDL, increased triglycerides, lower hemoglobin, women, diabetes mellitus, coronary artery disease but these factors were not consistent across studies[14,17,19,21,23] and since aspirin resistance was not a persistent finding in our patients the implications of finding a risk factor after a single measurement is unclear.

Despite preliminary evidence of an aspirin like antiplatelet effect of TCT, we did not find that TCT decreased the number of times patients on aspirin were resistance or had an aspirin effect compared to PBO in patients taking clopidogrel alone in our stroke, TIA population This may be explained by the fact that the pilot study involved small numbers of patients and the antiplatelet effect was not as robust as aspirin. We did, however, find a statistical decrease in the number of times aspirin resistance was detected with TCTs in patients on dual antiplatelet therapy with aspirin and clopidogrel, however, the significant result was due to the unusually high number of times aspirin resistance was seen in the PBO group receiving aspirin and clopidogrel, 40%, compared to all the other treatment groups receiving aspirin. Since aspirin dose may play a role in the incidence of aspirin resistance as mentioned above, if more patients in the PBO group were receiving 81 mg of aspirin rather than 325 mg than in the 400 mg, or 800 mg TCT groups that might explain the high incidence of aspirin resistance in the PBO group. However, in both the 400 mg, and 800 mg TCT groups more patients were taking 81 mg than in the PBO group, (18/22, (82%), 18/20 (90%), 22/38 (58%), respectively, $P = .02$, Chi square test). In patients taking aspirin and clopidogrel there is no obvious reason why taking clopidogrel should increase the incidence of aspirin resistance. Velik-Salchner et al did not find any differences in the percent of inhibition to arachidonic acid using LTA in patients a day after receiving a dose of 100 mg of aspirin and those receiving 100 mg of aspirin and 75 mg of clopidogrel.[24] Yet the frequency of aspirin resistance in the PBO group in patients taking aspirin and clopidogrel is higher at baseline and all of the follow-up visits in the 400 mg TCT treatment group and two of the four follow-up visits in the 800 mg TCT group. This suggests the higher incidence in the PBO group may be real and that despite the lack of an additive effect of TCT in patients on aspirin alone and no aspirin like effect of TCT in patients treated with clopidogrel alone, that TCT may have an additive effect on aspirin in patients who are also taking clopidogrel. Since the numbers are small, whether clopidogrel does in fact increase the incidence of aspirin resistance and whether TCT attenuates this response should be considered only hypothesis generating and warrants further study.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

C.K. Sen, A. Slivka, and C. Rink designed the research; A. Slivka, C. Rink, D. Paoletto, and C.K. Sen acquired and analyzed the data; A. Slivka, C. Rink, and C.K. Sen drafted a significant portion of the manuscript or figures. The manuscript has been read and approved for submission to FASEB J by all authors.

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