

Calcification in coronary artery disease can be reversed by EDTA–tetracycline long-term chemotherapy

Benedict S. Maniscalco^a, Karen A. Taylor^{b,*}

^a 4730 N. Habana Avenue, Suite 201, Tampa, FL 33614, USA

^b PA-C 2727 W. Martin Luther King Blvd., Suite 850, Tampa, FL 33607, USA

Received 7 May 2004; accepted 3 June 2004

Abstract

Atherosclerosis is a complex process with multiple mechanisms and factors contributing to its initiation and progression. Detection and quantification of coronary artery calcium (CAC) scores with electron beam tomography has been shown to correlate with obstructive and nonobstructive coronary artery disease (CAD). Pathogen-triggered calcification could play a role in CAD. Recent reports suggest that infectious blood nanobacteria (NB) emerge to be such a trigger. So far, minimal or no reversal of atherosclerosis has been claimed by therapies with *iv* ethylenediaminetetraacetic acid disodium salt (EDTA), antibiotics, or other regimens, and therapies for atherosclerosis remain non-curative. We have now combined EDTA with antibiotic tetracycline (comET), an *in vitro* proven nanobacteriocidal treatment, and tested comET therapy in patients with documented CAD. Three hypotheses were probed: (1) Are NB present in patients with CAD?; (2) Does treatment with comET affect blood NB antigen and serology?; (3) Does a comET decrease CAC scores?

One hundred patients with stable CAD and positive CAC scores were enrolled into a 4 month study of comET therapy. ComET therapy is composed of (1) Nutraceutical Powder (Vitamin C, Vitamin B6, Niacin, Folic Acid, Selenium, EDTA, L-Arginine, L-Lysine, L-Ornithine, Bromelain, Trypsin, CoQ10, Grapeseed Extract, Hawthorn Berry, Papain) 5 cm³ taken orally every evening; (2) Tetracycline HCl 500 mg taken orally every evening; (3) EDTA 1500 mg taken in a rectal suppository base every evening. CAC scoring was repeated at 4 months and serum samples were analyzed for NB antigen and serology at baseline, 2 and 4 months. Complete blood count, metabolic panel, liver function, C-reactive protein (hs-CRP) and lipids were analyzed at baseline and 4 months.

Seventy-seven patients completed the study and all patients were positive for NB serology, antigen or both. Responders ($n = 44$; 57%) had significant decreases in total CAC scores ($P = 0.001$), the average decrease being 14%. Non-responders ($n = 33$; 44%) had no change or had increases in CAC scores. Angina was decreased or ablated in 16 of 19 patients (84%). Lipid profiles improved to non-atherogenic direction significantly ($P = 0.001$), a remarkable finding in a patient group where 86% were on continuous statin medication already before the trial. No adverse physiologic effects were seen in renal, hepatic, or hematopoietic systems.

In conclusion, CAC scores decreased during ComET therapy trial in most CAD patients inferring regression of calcified coronary artery plaque volume. The patients tolerated the therapy well and their angina and lipid profiles improved. Further treatment trials for long term therapy with matched controls are warranted.

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Keywords: Coronary artery disease; Infection; Nanobacteria; Calcification

1. Introduction

Bench and clinical research has established that atherosclerosis is an inflammatory disease with injury or infection in the vascular endothelium predisposing to atheromas [1]. Inflammatory cascades wax and wane within

individual atheromas as the immune system attempts to “wall off” or isolate the area of injury [1,2]. The hallmark of this process is fibro-lipid matrix synthesis and degradation–absorption processes in soft plaque. O’Brien et al. [3] and Tenaglia et al. [4] demonstrated neovascularization of the atheroma at this stage of healing and plaque regression. These processes occur on a dynamic continuum within the atheromatous plaque. The rate of plaque synthesis–resorption is dependent upon the degree and/or stage of inflammatory activity within atheroma [4,5].

* Corresponding author. Tel.: +1 813 264 2241; fax: +1 813 265 5512.

E-mail address: ktaylor@nanobacslabs.com (K.A. Taylor).

Mature atheromas contain pathological calcification deposits that increase at an annual rate of 24–82% [6–9]. Quantification of coronary artery calcium (CAC) by computer tomography appears to be a better predictor of future events than conventional risk factors [10]. Although calcific deposits are a hallmark of atherosclerosis, the mechanism of calcium precipitation has remained enigmatic. Some researchers have suggested a relationship to the inflammatory cascade [1]. Infectious burden theory has linked many bacteria and virus to atherosclerosis but not with agents causing calcification in the host. Intriguingly, association of serum antibodies to mycobacterial heat-shock protein 65 with CAC scores suggests a role for pathogen-triggered calcification [11]. Although *Mycobacteria* are known inducers of calcification in the host, they have not been found in the plaques, and autoimmune reaction has been proposed as the cause for antibody formation. It remains to be elucidated what agent could trigger such antibodies in atherosclerotic patients.

Infection by unconventional apatite-forming bacteria-like nano-organisms, NB, is a novel theory for pathological calcification in general [12], and especially in CAD [13–17]. The latter role is supported by detection of nanobacteria (NB) or similar structures in atherosclerotic plaques [17], in calcified carotid arteries, aortic aneurysms and cardiac valves [18,19]. Furthermore, NB particles morphologically and functionally resemble the calcifiable vesicles, capable of active calcium phosphate precipitation under suitable nutrient conditions, previously isolated from atherosclerotic aorta [20].

NB are nanometer-sized, ubiquitous, pleomorphic agents that adapt to extremes of environment. They have a novel cell-wall structure and produce lipopolysaccharide (LPS) endotoxin biofilm [21]. The NB biofilm contains calcium apatite and prothrombin complex [16]. NB can be clinically detected with specific culture and electron microscopy techniques [13], and there exists a commercial ELISA test for nanobacteria antigen and serology (Nanobac Life Sciences, Inc., USA). Nanobacteria are sensitive *in vitro* to tetracycline and its action is increased by ethylenediaminetetraacetic acid disodium salt (EDTA) dissolving NB apatitic protective coat [22]. Thus, combination of these drugs might offer a novel treatment for calcific atherosclerotic disease. The present trial treatment regimen also included oral powder containing EDTA plus amino acids, vitamins and proteins to support the EDTA–tetracycline therapy and to elicit beneficial effects on known risk factors for heart disease.

The purposes of this investigation were: (1) to identify the serological presence of NB in a cohort of patients with CAD; (2) to evaluate possible changes in NB antigen and serology during antinobacterial (nanobiotic) comET therapy; and (3) to determine CAC score changes during the therapy.

2. Materials and methods

The treatment was applied on 100 patients presenting with stable coronary atherosclerotic heart disease who showed

positive CAC scores using Helical Computerized Tomography or Electron Beam Computerized Tomography[®]. All patients provided written, informed consent and were enrolled in this prospective, open-label observational study, and were treated with comET. Study inclusion criteria included: medically co-operative stable patients with documented CAD and positive CAC scores. Exclusion criteria included: (1) known tetracycline allergy; (2) zero CAC score; (3) recent (less than 30 days) major adverse cardiac event; (4) women of child-bearing age; (5) recent diagnosis of thyroid or parathyroid disease; (6) clinically significant renal insufficiency or liver function abnormalities and (7) recent (less than 30 days) acute congestive heart failure. The clinical protocol for this study was reviewed and approved by the Western Institutional Review Board (WIRB-Seattle, WA) on 6 December 2001.

One patient withdrew secondary to a presumed sensitivity to Tetracycline HCL. Twenty-two patients were withdrawn due to noncompliance.

All patients in the study were maintained on their normal medical regimen, but they were instructed to discontinue all herbal or vitamin preparations. Baseline history and physical examination were performed.

2.1. Nanobiotic therapy

ComET is a proprietary prescription compound formulated to treat atherosclerotic plaque forming processes potentially related to nanobacterial infection. ComET is composed of: (1) Nutraceutical Powder (Vitamin C, Vitamin B6, Niacin, Folic Acid, Selenium, EDTA, L-Arginine, L-Lysine, L-Ornithine, Bromelain, Trypsin, CoQ10, Grapeseed Extract, Hawthorn Berry, Papain) 5 cm³ taken orally every evening; (2) tetracycline HCl 500 mg taken orally every evening; (3) ethylenediaminetetraacetic acid disodium salt (EDTA-sequestant) 1500 mg taken in a rectal suppository base every evening. Study patients were instructed to comply with the following nightly dosing schedule: (1) mix the nanobiotic powder into water or apple juice and consume orally; (2) take the tetracycline HCl 500 mg capsule orally; (3) insert 1 suppository rectally and (4) lie down flat and go to sleep.

2.2. CAC scoring

The same CAC scoring machine was used for each individual patient to assess initial and final CAC scores. CAC scoring radiologists were experienced in CAC scoring and were blinded to patient identity. CAC scoring was repeated after 4 months of comET therapy.

2.3. Laboratory analysis

Study-patient blood specimen tubes were bar-coded and laboratory analysis were performed at a core independent clinical laboratory. The pathologist was blinded to patient

identity. The analysis included C-Reactive Protein (hs-CRP), Complete Blood Count (CBC), Metabolic Panel (CMP), Liver Functions Tests (LFT), Lipid Profile (LP), and NB ELISA antigen and NB IgG antibody serology (Nanobac Life Sciences, Inc., USA). NB serology was assessed at baseline, 2 and 4 months.

2.4. Statistical analysis

Data are presented as frequency and percentage distributions. Values of continuous variables are expressed as mean \pm standard deviation. Within group comparison of means for initial and ending CAC scores and laboratory values were conducted using a paired *t*-test. Between groups comparison of continuous variables was accomplished with the Student's *t*-test. Univariate analysis of selected discrete variables was accomplished by χ^2 , the continuity χ^2 analysis or a two-tailed Fischer Exact test with the appropriate degrees of freedom. Statistical procedures were performed using the *Number Cruncher Statistical Systems*[®] (NCSS, Kaysville, UT). A *p*-value of less than or equal to 0.05 was designated as statistically significant in the present study.

3. Results

3.1. CAC scores

The clinical characteristics of the final sample ($n = 77$) are described in Table 1. There were 44 (57%) patients who responded to therapy as evidenced by a decrease in total CAC score. The remaining 33 patients (43%) were considered non-responders only with reference to the CAC score parameter. Clinical variables for both groups are presented in Table 2. These data reveal that both groups were comparable with respect to pre-treatment clinical variables and risk factors. In Table 3, a comparison of initial and ending CAC scores for responders is presented. Total CAC scores decreased significantly ($P = 0.001$). Significant reduction in left anterior descending and right coronary artery CAC scores were also documented ($P = 0.002$). There was no significant difference found in the left main ($P = 0.972$) or circumflex coronary artery scores ($P = 0.106$).

3.2. NB antigen and antibodies

All patients were found to be positive for the presence of anti-NB IgG antibodies prior to the commencement of therapy (Table 4). During the course of therapy, NB antigen and serology titers tended to fluctuate, although changes were not statistically significant, in all patients without regard to changes in CAC scores or time-point of therapy, see Table 4. Initial and ending hs-CRP values were obtained in 19 patients. The values were relatively low, and they did not differ in the beginning and ending of therapy (0.54 ± 0.76 mg/l versus 0.45 ± 0.71 mg/l, $P = 0.674$).

Table 1
Characteristics of the CAD study population

Variables	Study participants
Total number	77 (100.0) ^a
Gender	
Male	62 (80.5)
Female	15 (19.5)
Age (years)	
Mean	63.2 \pm 8.9
Range	42–81
Age groups (years)	
Under 50	4 (5.2)
50–59	25 (32.5)
60–69	25 (32.5)
70–79	20 (26.0)
80 and over	3 (3.9)
Coronary risk factors	
Hypertension	52 (67.5)
Hyperlipidemia	68 (88.3)
Diabetes mellitus	20 (26.0)
Peripheral vascular disease	10 (13.0)
History of congestive heart failure	5 (6.5)
Renal insufficiency (Creatinine >2.0 mg/dl)	2 (2.6)
Previous myocardial infarction	14 (18.1)
Stable angina	19 (24.7)
Previous cardiovascular intervention	
Prior coronary artery bypass grafting	39 (50.7)
Prior percutaneous coronary intervention	17 (22.1)
Current medications	
Statins	66 (85.7)
Nitrates	23 (29.9)
Anticoagulants	3 (3.9)
Beta blockers	42 (54.6)
ACE inhibitors	28 (36.4)
Diuretics	20 (26.0)
Antiplatelets	55 (71.4)
Calcium blockers	12 (15.6)
ARB	18 (23.4)

^a Numbers in parentheses are percentages.

3.3. Lipid profiles

Beneficial changes were noted in the patients' lipid profiles as evidence by reduced total cholesterol levels ($P = 0.001$), reduced triglycerides ($P = 0.006$), decreased LDL ($P = 0.001$) and increased HDL ($P = 0.001$), see Table 5.

3.4. Angina pectoris

Prior to trial, 19 patients (25%) had stable angina pectoris. At the conclusion of 4 months of therapy, angina symptoms had been either substantially ameliorated or eliminated in 16 of 19 patients (84%: $P = 0.013$). Two patients (3%) with severe claudication and faint pedal pulses reported near resolution of symptoms with peripheral pulses returning to normal.

One patient (1%) was hospitalized with progressive angina secondary to an in-stent restenosis. All patients with

Table 2
Comparison of pre-treatment clinical variables and risk factors of study population in comET responder and nonresponder CAD patients

Variables	Responders ^a	Non-responders ^a	P value
Total number	44 (100.0)	33 (100.0)	
Gender			
Male	37 (84.1)	25 (75.8)	0.361
Female	7 (15.9)	8 (24.2)	
Age (years)			
Mean	64.9 ± 8.9	61.0 ± 8.4	0.058
Range	48 to 81	42–80	
Age groups (years)			
Under 50	2 (4.5)	2 (6.1)	
50–59	12 (27.3)	13 (39.4)	
60–69	14 (31.8)	11 (33.3)	
70–79	14 (31.8)	6 (18.2)	
80 and over	2 (4.5)	1 (3.0)	
Coronary risk factors			
Hypertension	31 (70.5)	21 (63.6)	0.527
Hyperlipidemia	41 (93.2)	27 (81.8)	0.160
Diabetes mellitus	11 (25.0)	9 (27.3)	0.822
Peripheral vascular disease	4 (9.1)	6 (18.2)	0.311
History of congestive heart failure	3 (6.8)	2 (6.1)	0.999
Renal insufficiency (Creatinine >2.0 mg/dL)	1 (2.3)	1 (3.0)	0.999
Previous myocardial infarction	10 (22.8)	4 (12.1)	0.370
Stable angina	16 (36.4)	3 (9.1)	0.013
Previous cardiovascular intervention			
Prior coronary artery bypass grafting	24 (54.5)	15 (45.5)	0.430
Prior percutaneous coronary intervention	10 (22.7)	7 (21.2)	0.999
Current medications			
Statins	39 (88.6)	27 (81.8)	0.515
Nitrates	16 (36.4)	7 (21.2)	0.151
Anticoagulants	2 (4.5)	1 (3.0)	0.999
Beta blockers	27 (61.4)	15 (45.5)	0.165
ACE inhibitors	15 (34.1)	13 (39.4)	0.632
Diuretics	12 (27.3)	8 (24.2)	0.764
Antiplatelets	32 (72.7)	23 (69.7)	0.771
Calcium blockers	5 (11.4)	7 (21.2)	0.238
ARB	8 (18.2)	10 (30.3)	0.214

^a Numbers in parentheses are percentages.

Table 3
Comparison of initial and ending scan CAC scores for comET responder CAD patients

Variables	Initial score ^a	Ending score ^a	P value
Total number	44 (100.0)	44 (100.0)	
Total score	2033.0	1753.8	0.001
Left main coronary artery	89.5	89.8	0.972
Left anterior descending coronary artery	927.7	788.4	0.002
Circumflex coronary artery	244.2	211.1	0.106
Right coronary artery	771.0	664.5	0.002

^a Numbers in parentheses are percentages.

compliance to treatment completed the protocol without serious adverse complications. Moreover, there were no other complications observed, e.g., non fatal myocardial infarction, cerebral vascular accident, in patient hospitalization or death during the course of the study. There

were minor side effects reported by the patients, such as gas and short-time diarrhea, and stomach pain during the therapy.

4. Discussion

This is the first study to combine antibiotic therapy with EDTA administration for a long-term therapeutic intervention in CAD patients undergoing optimal current therapy. The results were promising as every second CAD patient showed an objective improvement in their cardiac vasculature. CAC scores reduced by an average of 14% in a 4 months therapy trial. This is striking, because CAC scores are known to increase by more than 20% annually. There are no previous reports showing a significant decrease in CAC scores with any regimen found in literature. Thus, the novel therapy brought a unique, dramatic drop in hallmark for coronary events.

Table 4

Comparison of nanobacteria antibody levels (units) and antigen levels (units) for comET responder CAD patients responders

Variable	Beginning value mean \pm S.D.	Two month value mean \pm S.D.	Ending value mean \pm S.D.	<i>P</i> value
Nanobacteria antibody	0.864 \pm 0.35	0.954 \pm 0.48	0.981 \pm 0.50	
Beginning vs. 2 months				0.269
Beginning vs. ending				0.300
2 months vs. ending				0.799
Nanobacteria antigen	2.045 \pm 4.64	2.067 \pm 4.30	4.012 \pm 8.39	
Beginning vs. 2 months				0.982
Beginning vs. ending				0.206
2 months vs. ending				0.219

Since the two study groups, responders and non-responders, were comparable, it may be inferred that the variables of time, plaque density/volume, tissue penetration and blood supply may be critical factors that influence overall outcomes. Based on these findings, CAC scores should continue to decrease in this cohort with a longer duration of therapy. This was documented by substantial decreases in CAC scores in both responders and non-responders who elected to continue comET therapy following the conclusion of the study (data not shown).

Research with the pathogen NB, suggests that it can “trigger” atherogenesis, inflammation and thrombus formation [13–17]. The unique property of NB to utilize calcium (Ca^{2+}) and phosphate (PO_4^{4-}) to fix a calcium apatite layer upon its cell membrane at physiologic pH and concentration provides a viable explanation of the process of pathological calcification within the atheroma. NB gain entry into cells by molecular mimicry and endocytosis to establish a life-long parasitic relationship [23]. NB endotoxin [21] can cause acute inflammation and sustain chronic inflammatory cascades. Through these processes it is suggested that NB triggers inflammation resulting in deposition of fibro-lipid soft plaque and fibrous cap in addition to forming micro-calcifications.

In vitro studies have shown that EDTA decalcifies NB, but only at a sustained concentration. Further, tetracycline HCl is cidal to NB at pharmacologically acceptable and therapeutically achievable concentrations [22]. Based on these tenets, comET was formulated to be nanobacteriocidal.

If NB is present in patients with CAD, one would expect to document serologic evidence of their presence. This study documents the presence of NB in all patients. This study could not, however, detect significant variation in serology

during 4 month comET therapy. The therapy was reducing CAC scores in 44 patients (57%, $P = 0.001$). As the responders had 14% reduction in their CAC scores it meant that the therapy was not curing the calcifications in the 4 months period. Thus, it was unlikely that nanobacterial markers or hs-CRP could have markedly changed. Longer curative treatments would be needed to evaluate the marker responses.

ComET therapy may influence atherosclerosis in several ways that are independent from its actions on NB. EDTA is chelating calcium, copper and iron, high blood and tissue concentrations of which are suspected to promote atherogenesis through oxidative stress. EDTA chelates and removes via urine other poisonous heavy metals which may promote atherogenesis [24]. Interestingly, very high-dose (3 g per day) oral EDTA or subcutaneous EDTA-magnesium therapy have been reported to reduce cholesterol content in hypercholesterolemic rabbits [25,26]. Lipid modulating effects of EDTA are also supported by the present findings: comET therapy improved blood lipid patterns in CAD patients even under statin therapy. Increased activity of matrix metalloproteinases has been implicated in atherosclerosis in several ways. Metalloproteinase activity is dependent on zinc and calcium ions [27]. Both tetracycline and EDTA inhibit matrix metalloproteinases. EDTA and tetracycline also inhibit oxidative enzymes and act as antioxidants, even reducing experimental ischemic and reperfusion lesion sizes. EDTA has strong inhibitory action on blood clotting. EDTA may inhibit many calcium-mediated signaling pathways directly or indirectly via changes in the concentration of extracellular ionized calcium affecting function of calcium channels in cell membrane. One such target is immunological activation, another is smooth muscle contraction, both of importance, e.g., in coronary angina. All these action mechanisms could be pharmacologically important in atherosclerosis. Novel rectal administration of EDTA has been shown to result in high blood EDTA levels sustained for a long time (Kajander et al., manuscript in preparation). Furthermore, the contributory effects of the oral powder component should be evaluated. It contained several antioxidants, vitamins, amino acids among other agents. Thus, comET therapy is a multitargeted therapy focused on various aspects in atherogenesis. Further studies are needed to delineate its actions and targets.

Table 5

Comparison of beginning and ending cholesterol levels (mmol/l) for comET responder CAD patients

Lipid panel	Beginning value mean \pm S.D.	Ending value mean \pm S.D.	<i>P</i> value
Total cholesterol	4.9 \pm 1.2	4.2 \pm 0.88	0.001
Triglycerides	2.5 \pm 3.2	1.9 \pm 2.3	0.006
HDL cholesterol	1.2 \pm 0.319	1.4 \pm 0.34	0.001
LDL cholesterol	2.6 \pm 0.9	2.1 \pm 0.8	0.001

The present preliminary therapeutic results are encouraging. However, there are a number of inherent limitations to consider in this observational study. The primary limitation of the study is the lack of a control group. In addition, the small number of enrolled patients as well as those who withdrew from the study due to noncompliance with the nightly therapy detracts from the findings. Moreover, long-term outcome data must be collected to further support the results of the study.

5. Conclusion

ComET caused significant time-dependent decreases in CAC scores, thus inferring plaque regression. The presence of NB was identified serologically in all 77 patients. ComET did not produce any adverse physiological effects on study participants. Further clinical trials are warranted to address questions surfacing from this prospective observational study.

Acknowledgements

The author wishes to extend appreciation and thanks to: Dr. Olavi Kajander (Nanobac O.Y., Kuopio, Finland) and Neva Ciftcioglu (Universities Space Research Association, Johnson Space Center, Houston/TX) for their guidance and constructive critiques; Dr. George Ebra (statistical analysis); and Dr. Richard Berger (Miami Heart Institute, Miami, FL) and Dr. Alan Miller (University of Florida, Jacksonville) who served on the study oversight committee. The author acknowledges Drs. William S. Maxfield and Mark Herbst, for performing and interpreting CAC scans and scores.

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Table 5
 Comparison of Beginning and Ending Cholesterol Levels (mg/dl) for Responders

Lipid Panel	Beginning Value Mean \pm S.D.	Ending Value Mean \pm S.D.	<i>p</i> Value
Total Cholesterol	188.6 \pm 47.4	164.5 \pm 33.8	0.001
Triglycerides	232.0 \pm 302.5	179.4 \pm 221.1	0.006
HDL Cholesterol	47.4 \pm 12.1	52.2 \pm 13.0	0.001
LDL Cholesterol	101.8 \pm 35.6	81.3 \pm 29.5	0.001