EFFECT OF PREOPERATIVE SUPPLEMENTATION WITH α -TOCOPHEROL AND ASCORBIC ACID ON MYOCARDIAL INJURY IN PATIENTS UNDERGOING CARDIAC OPERATIONS

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Augmentation of antioxidant defenses may help protect tissues against ischemia-reperfusion injury associated with operations involving cardiopulmonary bypass. In this study we examined the effect of pretreating patients with α -tocopherol (vitamin E) and ascorbic acid (vitamin C) or placebo on injury to the myocardium. Seventy-six subjects undergoing elective coronary artery bypass grafting participated in a prospective, double-blind, placebo-controlled randomized trial, receiving either placebo or both 750 IU dl- α -tocopherol per day for 7 to 10 days and 1 gm ascorbic acid 12 hours before the operation. Plasma α -tocopherol concentrations, raised fourfold by supplementation, fell by 70% after the operation in the supplemented group and to negligible levels in the placebo group. There were no significant differences between the groups with respect to release of creatine kinase MB isoenzyme over 72 hours, nor in the reduction of the myocardial perfusion defect determined by thallium 201 uptake. Electrocardiography provided no evidence of a benefit from antioxidant supplementation. Thus the supplementation regimen prevented the depletion of the primary lipid soluble antioxidant in plasma, but provided no measurable reduction in myocardial injury after the operation. (*J Thorac Cardiovasc Surg* 1997;113:942-8)

Cardiac surgery with cardiopulmonary bypass (CPB) is associated with the risk of ischemia-reperfusion type injury to the myocardium and other tissues, as a result of aortic crossclamping and altered arterial blood flow patterns. Tissue injury may arise during both the period of ischemia and the initial seconds of reperfusion.¹ In terms of the free radical hypothesis, reperfusion injury results from the production of oxygen-derived free radicals and other reactive oxygen substances in amounts

that overwhelm the body's endogenous antioxidant defenses. The whole-body inflammatory reaction resulting from CPB contributes to the oxidative stress. Complement-activated neutrophils are a major source of oxidant species^{1,2} and are implicated in microvascular injury and consequent parenchymal organ injury.^{1,3}

Evidence for myocardial reperfusion injury induced by oxygen-derived free radicals has been largely derived from animal models and in vitro perfused hearts; studies in human beings are limited.⁴⁻⁸ Recently, it has been reported that myocardial reperfusion after aortic crossclamping in human beings leads to increases in markers of free radical activity, such as lipid peroxidation,⁹⁻¹¹ and a decrease in myocardial concentrations of the major membrane antioxidant α -tocopherol.¹² Studies showing a protective effect of allopurinol, an inhibitor of xanthine oxidase (which catalyzes the production of superoxide radicals) during coronary artery bypass grafting (CABG) are consistent with the notion of oxidative stress during reoxygenation.^{6,13,14}

There are a number of theoretic approaches to limiting the toxic effects of oxygen-derived free radicals in vivo.³ One is to augment the body's antioxidant defenses before surgery.¹⁵ The primary

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physiologic antioxidants are the lipid-soluble, chain-breaking vitamin α -tocopherol (vitamin E) and, in the aqueous phase, ascorbic acid (vitamin C) and glutathione.¹⁶ Initial studies suggest that pretreatment of patients undergoing CPB with α -tocopherol or ascorbic acid reduces lipid peroxidation (malondialdehyde production) and myocardial tissue injury (enzyme efflux) during CPB.^{8, 9, 17, 18} However, the potential benefit of antioxidant administration, and especially of α -tocopherol, on ischemia-reperfusion injury in vivo is controversial.

The present study was undertaken to determine the effect of pretreating patients undergoing CPB with both α -tocopherol and ascorbic acid or placebo on markers of myocardial injury after CPB. Inasmuch as α -tocopherol is relatively poorly absorbed, dietary supplementation was extended to 7 to 10 days before the operation to augment both its circulating and tissue concentrations.^{8, 19} Ascorbic acid, on the other hand, is readily absorbed and distributed to tissues. Because ascorbic acid may be involved in the regeneration of α -tocopherol from its α -tocopheroxyl radical,²⁰ supplementation with both antioxidants was used to maximize antioxidant protection after CPB.

Methods

Patients. Patients undergoing CABG were admitted to the trial if the following criteria were met: the patient was aged 18 to 75 years, did not smoke, and gave informed consent more than 10 days before the operation. Patients were excluded if they were receiving coumarin derivatives (very high doses of vitamin E may potentiate anti-vitamin K therapy), vitamin supplements, or corticosteroids. In addition, malabsorption syndromes, severe asthma, obstructive pulmonary disease, poor left ventricular function (ejection fraction < 40%), and a history of alcoholism, drug abuse, psychosis, or a combination of these conditions excluded patients from the study. Eligible patients were then assigned the next available (randomized) study number and accompanying coded treatment.

Seventy-nine patients were enrolled. Three subjects were excluded after randomization because of canceled operations (urgent cases took priority over scheduled operations); thus the study finally consisted of 76 patients. Thirty-eight patients were assigned to the vitamin-supplemented (active) group and the same number to the placebo group. The mean age was 59.9 years; 63 of 76 (82.9%) were male (Table I).

The study was conducted in accordance with the ethical guidelines of the National Health and Medical Research Council of Australia; the protocol was approved by the Human Ethics Committee of The Prince Charles Hospital, Brisbane.

Intervention (supplementation). Enrolled subjects received a 10-day supply of capsules (active or placebo) for

Table I. Clinical characteristics of patients

Parameter	Placebo	Supplemented	Combined
Age (mean $\pm$ SD; yr)	59.7 $\pm$ 7.7	60.0 $\pm$ 7.0	59.9 $\pm$ 7.3
Sex ratio (male/female)	30:8	33:5	63:13
Bypass time (median, first quartile, third quartile; min)	68.0	74.0	70.5
Crossclamp time (median, first quartile, third quartile; min)	50.8, 90.0	56.8, 107.0	55.0, 90.0
No. of grafts (1/2/3/4/5/6)	41.0	44.0	43.0
	29.5, 55.0	30.8, 55.3	30.0, 55.0
	1/5/11/10/8/1	0/2/12/12/9/2	1/7/23/22/17/3

SD, Standard deviation.

self-administration. The active capsules provided 750 IU dl- α -tocopheryl acetate daily; the placebo provided an equivalent amount of sucrose. Compliance was monitored by measuring serum α -tocopherol levels and by capsule counts. Twelve hours before the operation, supplemented patients also received 1 gm ascorbic acid orally, and control subjects received a placebo (1 gm sucrose). No side effects attributable to the supplement or placebo were reported.

Surgical procedure. Patients underwent CABG with standard CPB. In brief, after systemic heparinization, CPB was established with Cobe roller pumps (Cobe Laboratories, Inc., Denver, Colo.), a flat-sheet membrane oxygenator (Cobe) and an arterial filter (Pall Biomedical Products, East Hills, N.Y.). The extracorporeal circuit was primed with 2 L of crystalloid solution (lactated Ringer's solution); packed cells were included if the diluted hematocrit value was expected to fall below 0.20. Minimum flow rates of 2.4 L/min per square meter and core temperatures of 30° to 32° C were maintained throughout CPB. Multi-dose hypothermic potassium cardioplegic solution and topical hypothermia were used during aortic crossclamping. Median CPB and crossclamp times are shown in Table I.

Sample collection. Peripheral venous blood was collected before the operation and at intervals over the subsequent 72 hours.

Laboratory methods. Plasma creatine kinase isoenzyme MB (CK-MB) was estimated by electrophoresis by means of the REP CK-8 isoenzyme system (Helena Laboratories, Beaumont, Tex.). Plasma α -tocopherol was estimated by ultraviolet detection at 285 nm after separation by high-performance liquid chromatography.²¹ α -Tocopherol acetate served as an internal standard. Because fluid balance is largely restored by 12 hours and because our primary concern was the comparison between patient groups, plasma enzyme and vitamin levels were not corrected for the effect of the crystalloid pump prime.

Electrocardiography. Electrocardiograms were obtained before the operation and at hospital discharge (usually the sixth or seventh day). A 54-criteria QRS scoring system²² was used to evaluate the extent of myocardial damage. The theoretic maximal score on this system is 32 points, with each additional point representing a loss of approximately 3% of left ventricular myocardium. Measurements were made on standard 12-lead

Fig. 1. Changes in plasma α -tocopherol concentrations after cardiac surgery (medians and first and third quartiles). The *open circles* indicate patients receiving supplemental α -tocopherol and ascorbic acid, and the *solid symbols*, the placebo group. The difference between groups, changes with time, and group $\times$ time interaction were all highly significant ($p < 0.001$).

electrocardiograms recorded at a frequency response of 0.05 to 100 Hz using manual callipers.

Thallium scans. The extent of perioperative segmental myocardial injury was assessed by single-photon emission computed tomographic (SPECT) thallium 201 (Tl-201) scans obtained at rest in the preoperative period and before hospital discharge. Patients underwent the scan if they gave written, informed consent and if the scan could be scheduled during the work week ($n = 41$). Subjects were given an intravenous injection of 80 MBq of Tl-201 at rest after an overnight fast. Within 30 minutes of injection, a SPECT scan was acquired in 32 angles over 180 degrees (right anterior oblique to left posterior oblique). Tomograms were reconstructed by filtered back-projection, with oblique-angle reorientation along the long axis of the left ventricle, and displayed in oblique short-axis and horizontal and vertical long-axis slices. Preoperative and postoperative scan pairs for each patient were compared in the same format as conventional stress/rest Tl-201 scan pairs, with quantitation of myocardial thallium distribution using a two-dimensional polar map.²³ Scans were interpreted by an experienced nuclear medicine physician who was blinded to the patient's treatment.

Total defect extent was estimated from the polar map analysis, as the percentage of total left ventricular volume in which Tl-201 uptake (relative to maximal uptake) was more than 2.5 standard deviations below the mean of a gender-specific reference database of resting myocardial

Tl-201 distribution. Defect severity on the tomographic slices was graded for each of five segments (anterior, septal, inferior, lateral, and apical) using a semiquantitative color table, as follows: 0 = normal; 1 = mild ($> 80\%$ of maximal uptake)/questionable defect/probable artifact; 2 = definite defect (50% to 80% of maximal uptake); 3 = severe ($< 50\%$ of maximal uptake). The total score for all five segments was used as a global severity score. In addition, a combined (severity $\times$ extent) score was determined.

Statistics. The present trial of preoperative supplementation with antioxidant vitamins in cardiac surgery involved a renal as well as a cardiac arm; the renal substudy will be reported elsewhere. Sample size determinations and the power calculations were based on renal outcomes. On the basis of log transformed data from the placebo group ($n = 38$), retrospective power calculations showed that a 75% change in the logged CK-MB area under the curve would be detected with power = 80% and $\alpha = 0.05$ (two-tailed). As a result of substantial skewness of the distributions, values are reported as medians (first quartile, third quartile). Pre-post differences were approximately normally distributed, and consequently differences between the patient groups' time-related data were analyzed by parametric paired t tests or two-way repeated-measures analysis of variance, with the use of SPSS-PC+ version 4.0 (SPSS, Inc., Chicago, Ill.). A square root transformation was applied to CK-MB time profiles, QRS scores, and to α -tocopherol values because of unequal variances across the groups. For the calculation of the areas under the CK-MB plasma concentration curves, Sigmaplot graphic software (Jandel) was used, with the trapezoidal rule used for unequally spaced x values.

The significance of differences between two data sets was determined by Student's t test or the Mann-Whitney rank sum test for parametric and nonparametric data, respectively. Correlations between variables were sought with the use of the Pearson product moment correlation. A p value < 0.05 was considered significant.

Results

There were no differences between the study groups with respect to duration of bypass ($p = 0.37$) and total crossclamp times ($p = 0.60$). There was no significant difference in the proportion of patients with crossclamp times greater than 50 minutes (0.36 in the supplemented group; 0.30 in the placebo group; $p = 0.75$). One patient required surgical débridement and rewiring for sternal infection, and another was reintubated and the lungs were ventilated on day 5. One patient did not regain consciousness and died after the study period.

Plasma α -tocopherol concentrations (Fig. 1). Preoperative plasma α -tocopherol concentrations were fourfold higher in the supplemented group (medians 44.7 vs 11.4 mg/L, $p < 0.001$). Postoperatively, median concentrations in the supplemented group fell by 70% but remained at or above normal

Fig. 2. Changes in plasma CK-MB concentrations after cardiac surgery (medians and first and third quartiles). Laboratory normal reference range, 0 to 10 U/L. The changes with time were highly significant ($p < 0.001$); group $\times$ time interaction, $p = 0.47$; difference between groups, $p = 0.71$.

Table II. CK-MB release after cardiac operations

Parameter	Placebo group (n = 36)		Supplemented group (n = 37)		p Value
	Median	First, third quartile	Median	First, third quartile	
CK-MB peak (U/L)	29	16, 53	28	17, 43	0.81
Area under curve (U $\cdot$ hr/L)	622	333, 793	476	299, 902	0.56

levels. In contrast, concentrations in the placebo group decreased from normal to negligible levels within 3 hours of the operation. The difference between groups, changes with time, and group $\times$ time interaction were all highly significant ($p < 0.001$).

Plasma creatine kinase isoenzymes (Table II and Fig. 2). Changes in plasma CK-MB concentrations with time were highly significant ($p < 0.001$). Median peak values reached were 29 and 28 U/L in the placebo and supplemented subjects, respectively ($p = 0.81$). There were no significant differences between the groups with respect to CK-MB profiles ($p = 0.71$) nor with respect to CK-MB areas under the curve ($p = 0.56$). The median of the placebo group (622 U $\cdot$ hr/L), however, was 23.5% higher than in the supplemented group (476 U $\cdot$ hr/L). The power of the performed test was, however, low (0.37), suggesting that this negative finding be interpreted with caution.

There was no correlation between preoperative plasma α -tocopherol levels and the CK-MB area under the curve for the two patient groups considered either separately or collectively or when the combined data were log-transformed ($|r| < 0.1$, $p > 0.7$).

Electrocardiography (Table III). Adequate paired preoperative and postoperative electrocardiograms were available in 49 patients (23 placebo, 26 active treatment). Reasons for exclusion from the electrocardiographic analysis included the presence of bundle branch block ($n = 12$) or left ventricular hypertrophy ($n = 1$), inadequate technical quality for analysis ($n = 8$), or death ($n = 1$).

The active treatment group had significantly worse QRS scores than the placebo group at baseline ($p = 0.03$), presumably as a result of chance. Postoperative QRS scores were worse than preoperative scores in the combined group ($p = 0.001$), although the interaction between group and time

Table III. Extent of myocardial injury determined by QRS score (units)

	Placebo group (n = 23)		Supplemented group (n = 26)	
	Median	First, third quartile	Median	First, third quartile
Preoperative	0	0, 1	1	0, 3
Postoperative	1	0, 2	4	1, 8
Change	0	0, 2	0	0, 3

Between-group comparison: $p = 0.008$; changes with time: $p = 0.001$; group $\times$ time interaction: $p = 0.92$.

was not statistically significant ($p = 0.92$). There was no difference between the two groups with respect to the proportion of subjects with new Q waves after the operation (0.33 and 0.36 for placebo and supplemented groups, respectively; $p = 0.92$).

Thallium scans (Table IV). There were no significant differences between the groups before the operation or at the time of discharge with respect to either the severity or the extent of perfusion defects at rest or for the combined (severity $\times$ extent) score. As expected, a significant improvement in all these parameters was noted after CABG ($p \leq 0.002$).

Discussion

As the primary hydrophobic, chain-breaking antioxidant, α -tocopherol has attracted interest as an agent for limiting ischemia-reperfusion injury resulting from oxygen-derived free radicals.^{8, 11, 12, 17} Studies of α -tocopherol treatment (frequently combined with ascorbic acid) in isolated perfused animal hearts have consistently shown less functional, structural, and biochemical changes than in controls.²⁴⁻²⁶ In human studies, plasma and myocardial α -tocopherol levels have been shown in some^{8, 11, 12, 17} but not all studies⁷ to decrease during bypass. This fall may be prevented by supplementation.¹⁷ Supplementation also attenuated the rise in levels of plasma hydrogen peroxide (H_2O_2)¹⁷ and the lipid peroxidation marker malondialdehyde during and immediately after CPB.⁹ Whether these effects were clinically significant was addressed in the present study, which showed that augmentation of patients' antioxidant reserves by supplementation with α -tocopherol and ascorbic acid had no significant effect on markers of myocardial injury after uncomplicated CPB operations. The lower median for CK-MB area under the curve in the treated group, although not statistically significant, is of a magni-

tude (23.5%) to suggest a possible clinical effect in some patients.

The lack of a demonstrable protective effect in the present study cannot be attributed to low concentrations of α -tocopherol. Although plasma levels and not tissue levels were measured, it is probable that the latter was raised albeit to a lesser degree.⁸ After surgery, plasma concentrations of α -tocopherol fell by 70% in the supplemented group, but concentrations remained at or above normal levels (see Fig. 1). In contrast, concentrations in the placebo group fell to negligible levels within 3 hours of the operation. Although part of this decrease may be attributed to hemodilution, the observed changes far exceeded the effects of hemodilution. Part of the change may also be attributed to changes in plasma lipids. Because the bulk of the α -tocopherol is carried by plasma lipoproteins, levels of the vitamin are highly correlated with total plasma lipid levels,⁷ which tend to fall in the postoperative period. Nevertheless, the data indicate a clear difference between the groups in the circulating levels of α -tocopherol after surgery.

Studies in isolated perfused animal hearts²⁴⁻²⁶ suggest that the fall in antioxidant reserve may have exposed some patients to the risk of clinically significant myocyte injury in the postoperative period. CK-MB profiles were fairly similar in supplemented and placebo groups; however, the power of the performed test for CK-MB area under the curve (normalized data) was low (0.37), suggesting that the negative finding be interpreted with caution. It is therefore not possible to exclude a clinically significant benefit with certainty. The 23.5% lower median for CK-MB area under the curve in the supplemented group is suggestive of a clinically significant effect, although changes of less than 25% are unlikely to be of major functional or prognostic significance. It is possible that a larger clinical trial may detect a clinically and statistically significant effect. Earlier studies are not helpful in this respect. On the basis of their study of 18 patients, Ballmer and associates⁷ concluded that α -tocopherol (and carotenoid) concentrations were of no major concern during and after cardiac operations. In another recent study involving use of α -tocopherol supplementation or placebo in 28 patients with cardiac disease, Yau and colleagues⁸ showed small but significant improvements in myocardial metabolism, ventricular function, and CK-MB release but concluded that these changes were not clinically significant for low-risk patients.

Table IV. Extent and severity of cardiac defect before and after cardiac surgery as determined by TI-201 scans

Parameter	Placebo group (n = 18)		Supplemented group (n = 23)	
	Median	First, third quartile	Median	First, third quartile
Extent (%) [*]				
Pre	14.0	2, 28	18.0	7, 40
Post	4.5	4, 14	3.0	1, 29
Severity (score) [†]				
Pre	3.0	1, 6	3.0	2, 10
Post	1.0	0, 3	2.0	1, 7
Severity × extent (score) [‡]				
Pre	28.0	4, 190	36.0	16, 420
Post	9.0	0, 34	9.0	1, 273

^{*}Between-group comparison: $p = 0.45$; changes with time (pre-post): $p = 0.001$; group × time interaction: $p = 0.98$.

[†]Between group comparison: $p = 0.09$; changes with time (pre-post): $p = 0.001$; group × time interaction: $p = 0.22$.

[‡]Between group comparison: $p = 0.12$; changes with time (pre-post): $p = 0.002$; group × time interaction: $p = 0.45$.

A note of caution may be added here. None of the patients in the present study had low levels of α -tocopherol in the preoperative period; it is possible that preoperative deficiency of the antioxidant may have yielded different results. Second, the present group of patients undergoing CABG were subjected to relatively short periods of ischemia (median crossclamp time 43 minutes). Prolonged ischemic periods are more likely to be associated with oxidative stress.²⁷ Third, although CK-MB is the traditional gold standard for the diagnosis of myocardial infarction, peak values may be misleading after cardiac surgery.²⁸ We therefore combined CK-MB measurements with other myocardial indicators (electrocardiograms and TI-201 scans). It is possible that the latter investigations may not have found significant group differences, because they are not sensitive indicators of perioperative free radical injury. They have also not been specifically validated in a cardiac surgical population. Interpretation of the QRS scoring was complicated by the baseline imbalance between the treatment and placebo groups, with the treatment group having more extensive damage before surgery. However, QRS scores increased by a similar extent in both the placebo and treatment groups, with no suggestion of a benefit from antioxidant supplementation.

The efficacy of high-dose ascorbic acid administration on myocardial reperfusion injury in human beings has been recently reported. Intravenous ascorbic acid (1 gm) 90 minutes before bypass followed by cardioplegic solutions containing ascorbic acid (1 mmol/L) significantly reduced the release of cardiac enzymes in patients with ischemic periods greater than 50 minutes.²⁹ High doses of ascorbic acid (250 mg/kg) given intravenously before CPB

and at aortic crossclamping significantly reduced serum malondialdehyde, CK-MB, and lactate dehydrogenase concentrations in the postoperative period.¹⁸ Cardiac output was significantly higher in the treated patients for the first 6 hours after the operation.¹⁸ These two studies suggest that high doses of ascorbic acid may effectively scavenge free radicals during and after CPB and offer a measure of protection to the myocardium. No statistically significant effects were seen in the present trial, in which an effectively lower dose was used (1 gm orally 12 hours before the operation).

The lack of obvious benefit from antioxidant pretreatment in the present study is disappointing in the light of numerous animal studies that support the concept of ischemia-reperfusion injury amenable to protection by antioxidants.²⁴⁻²⁶ Whether this is due to species differences, differences in treatment regimens, or periods of induced ischemia is uncertain. In human studies, administration of drugs with known antioxidant activity may be confounding. Allopurinol, β -blockers, and calcium channel blockers (which were commonly administered before surgery) may independently contribute to the free radical scavenging defenses of the patients.^{8, 10, 13} Concomitant medications were similar in the patient groups, and drug-related effects are therefore unlikely to be a confounding factor in the present study.

In summary, extended supplementation with α -tocopherol combined with ascorbic acid 12 hours before routine, uncomplicated cardiac surgery prevented the depletion of the primary lipid-soluble antioxidant α -tocopherol in plasma, but provided no additional protection to the myocardium over the standard surgical regimen. Further studies are re-

quired to determine the possible benefit for patients with longer crossclamp times who are at greater risk of ischemia-reperfusion injury.

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