Mobilization of Antioxidant Vitamin Pools and Hemodynamic Function After Myocardial Infarction

Vince P. Palace, PhD; Mike F. Hill, PhD; Firoozeh Farahmand, MD; Pawan K. Singal, PhD, DSc

Background—Although most previous studies have attempted to correlate plasma concentrations of vitamins with specific cardiovascular end points, metabolic considerations suggest that changes in myocardial tissue and storage organs may be better indicators of myocardial oxidative stress.

Methods and Results—Rats fed commercial chow or a diet enriched with vitamin E for 2 weeks were subjected to either a surgical myocardial infarction (MI) or a sham procedure. Rats were hemodynamically assessed 16 weeks after surgery, and their heart, liver, kidney, and plasma were analyzed for antioxidant vitamins E (tocopherol) and A (retinol and total retinyl esters). At 16 weeks, MI rats on a control diet showed depressed peak systolic and elevated diastolic pressures in both right and left ventricles compared with their sham controls. Plasma concentrations of vitamins E and A in MI rats were not different from sham controls fed the same diet. However, concentrations of vitamin E in left ventricle and liver and of vitamin A in liver (retinol) and kidney (retinyl esters) were decreased in rats with MI compared with the sham controls. Vitamin E supplementation improved hemodynamic function in rats with MI and increased plasma, myocardial, liver, and kidney concentrations of vitamin E. The vitamin E diet also prevented the loss of total retinyl esters from the kidney but not of retinol from the liver in MI rats.

Conclusions—Dietary supplements of vitamin E can sustain better cardiac function subsequent to MI. Antioxidant vitamin levels in the myocardium or in storage organs and not in plasma may be better indicators of myocardial oxidative stress. (Circulation. 1999;99:121-126.)

Key Words: heart failure • free radicals • myocardial infarction • antioxidants

Current interest in the antioxidant vitamins E and A as they relate to heart disease has evolved from the growing realization that free radical–mediated tissue damage is involved in the pathogenesis and progression of several forms of cardiac dysfunction. Several large-scale epidemiological studies have recently shown an inverse relationship between vitamin E and A consumption and the risk of cardiovascular disease. Furthermore, experimental evidence has shown depletion of these antioxidant vitamins in different cardiac abnormalities. However, there is also experimental evidence suggesting that plasma and/or myocardial concentrations of vitamins E and A are not related to oxidative stress or depressed cardiac function. Clearly, more work is required to clarify the role of antioxidant vitamins in heart failure.

In the majority of past studies, plasma was the target site for analysis of vitamin concentrations. A few studies have also considered vitamin concentrations specifically in heart tissue. The myocardium and resident blood plasma are certainly expected to be the tissues initially affected by free radical production during cardiac disturbances. However, the heart contains only a small proportion of the body’s total antioxidant vitamin pool. Larger stores, present in the liver and kidney, are thought to function as reservoirs. Therefore, potential increases in the utilization of antioxidant vitamins in the heart and plasma after a disruptive cardiac event may be buffered by mobilization of vitamins from other larger storage pools in the body. In fact, in animals exposed to chemicals that alter vitamin A metabolism, liver and kidney have been reported to be more sensitive indicators of vitamin A status than serum concentrations.

In the present study, antioxidant vitamin E and A concentrations in the plasma, myocardium, liver, and kidney were measured in hemodynamically assessed rats with or without myocardial infarction (MI). The effects of dietary vitamin E supplementation for 16 weeks on vitamin E and A status, as well as hemodynamic function in these groups, were also examined.

Methods

Animals and Surgical Treatment

Male Sprague-Dawley rats (125±7 g; n=60), housed in pairs, were randomly assigned to either a basal diet (rat chow, PMI feeds) or a vitamin E–enriched diet (1545 mg of tocopherol acetate per kilogram of feed). After 2 weeks on these diets, each group was further...
Antioxidant Vitamins After MI

subdivided into an MI and a sham group (n=15 each). Rats in the MI groups were anesthetized and underwent surgery to ligate the left coronary artery, as described previously. Using this surgical procedure, we have previously shown that an infarct is produced that reaches ≤50% of the left ventricle. To reduce variability due to differences in infarct size, rats with infarcts of <20% of the left ventricular mass were not included in the present study. Rats in the control groups underwent the same procedure, except that the suture was not tied around the coronary artery. After recovering from the procedures, rats were kept on diets identical to their presurgical grouping for an additional 16 weeks.

At the end of 16 weeks, animals were anesthetized with sodium pentobarbital (50 mg/kg IP), and their left and right ventricular functions were assessed by a miniature pressure transducer. After these assessments, animals were killed, and the heart, liver, and kidney were removed and immediately frozen in liquid nitrogen. Plasma was isolated from whole blood by centrifugation and similarly frozen.

All of the animals used in the present study were maintained and treated in accordance with the policies and procedures of the Canadian Council on Animal Care.

Vitamin Measurement

Vitamin E (tocopherol) and vitamin A (retinol and retinyl esters) were measured in the myocardium, liver, kidney, and plasma, as well as in the feed, by use of an extraction procedure and reverse-phase high-performance liquid chromatography (HPLC) detection method.

Synthesis and Structural Confirmation of Vitamin A Esters

Because only the palmitate ester of Vitamin A is commercially available, HPLC standards for other vitamin A esters were synthesized by use of a modification of a cholesterol ester synthesis method. All preparatory steps were performed under subdued light conditions and in amber vials. Briefly, 2020 nmol of vitamin A (retinol) in toluene was placed by means of a pipette into a vial that contained 6060 nmol of the appropriate lipid anhydride, also suspended in toluene. Contents of the vials were thoroughly mixed. Each sample was completely dried under vacuum in a rotary evaporator at room temperature, purged with nitrogen, tightly capped, and incubated in the dark at 68°C in a water bath for 8 to 12 hours. Samples were then resuspended in HPLC mobile phase and analyzed by the HPLC method. Retention times for each unknown ester were established, and post-HPLC eluant fractions corresponding to these intervals were collected and dried under vacuum. After suspension in the HPLC mobile phase, the samples were eluted by the same HPLC separation procedure. Each eluant corresponding to a vitamin A ester peak retention time was analyzed by mass spectrometry to confirm the vitamin A ester in that peak.

Data Analysis

All data are presented as mean±SEM. Group means were analyzed by ANOVA followed by Bonferroni pairwise t tests to identify differences between specific means. Statistical significance was set at P<0.05.

Results

Hemodynamics

Results from the hemodynamic assessments of right and left ventricles from the 4 groups are summarized in the Table. Animals fed the basal diet in the 16-week post-MI group showed an elevation in left-ventricular end-diastolic pressure and a depression of left-ventricular peak systolic pressure compared with control rats fed the same diet. A similar pattern of pressure changes was present in the right ventricle. Control animals fed the diet supplemented with vitamin E showed no change in any of their hemodynamic parameters compared with animals on the basal diet. Vitamin E feeding significantly attenuated the rise in left-ventricular end-diastolic pressure as well as the drop in left-ventricular peak systolic pressure in the MI group, but both of the values were significantly different from control levels. Values for the right ventricle (end-diastolic and peak systolic pressures) in the vitamin E-supplemented MI group were maintained near control levels.

Vitamin E Consumption and Tissue Concentrations

Vitamin E content was 40.6±1.4 mg/kg in the basal diet and 1545±101 mg/kg in the enriched diet. Analysis of each diet at the beginning and end of the experiment determined that loss of vitamin E was <4% and was not significantly different between any batch of the diet preparations. There were no significant differences in the intake of vitamin E per day between the 2 basal diet groups (sham=1.15±0.02 mg/d per animal; MI=1.11±0.04 mg/d per animal) or the enriched-diet groups (sham=32.53±1.03 mg/d per animal; MI=32.03±0.9 mg/d per animal).

Vitamin E concentrations in plasma, left ventricle, right ventricle, liver, and kidney are shown in Figure 1. Vitamin E concentration was highest in the liver and left and right ventricles, followed by kidney and plasma. MI depleted tocopherol in the left ventricles by >32% and in the liver by 37% in rats fed the basal diet compared with their sham controls. MI in rats fed the basal diet had no effect on vitamin E levels in the plasma, right ventricle, or kidney.

The vitamin E–enriched diet significantly (P<0.05) elevated concentrations of the vitamin in all of the tissues from sham animals (Figure 1). The maximum percent increase was in the liver (340%), followed by plasma (250%), both ventricles (60%), and kidney (26%). A significant gain in vitamin E concentrations was also seen in all of the tissues in the supplemented MI animals. However, in the MI animals, left ventricular vitamin E concentration was significantly less (−25%) than the sham controls supplemented with vitamin E. There was a trend toward lower vitamin E concentrations in the liver of MI rats fed the enriched diet compared with their

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<tr>
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†Significantly (P<0.05) different from sham control group receiving the same diet.

‡Significantly (P<0.05) different from basal diet MI group.
diet controls, but the difference was not statistically significant.

Tissue Concentrations of Vitamin A (Retinol and Total Retinyl Esters)

Using synthesized and authenticated vitamin A ester standards, we performed analyses of different vitamin A esters in the heart, liver, kidney, and plasma tissues from rats fed the basal and enriched diets. Retinol was present in all tissues (liver, kidney, heart, and plasma). At least 5 retinyl esters were routinely detected in the liver (palmitate, stearate, oleate, linoleate, octanoate), 3 in the kidney (palmitate, stearate, octanoate), and only 1 (palmitate) in the plasma and the heart. Although retinyl palmitate was detectable in plasma and in both the right and left ventricles, its concentration was highly variable and was generally <0.002 nmol/g.

Retinol concentrations in the plasma, left ventricle, right ventricle, liver, and kidney are shown for all 4 experimental groups in Figure 2. Plasma retinol concentrations were unaffected by MI. Sham and MI rats fed the vitamin E–enriched diet had significantly higher concentrations of retinol in plasma than their respective basal diet controls. Neither dietary vitamin E supplements nor MI had any effect on left or right ventricular retinol concentrations. MI depleted liver retinol by 34% in rats fed the basal diet compared with their sham controls, and this depletion was not significantly attenuated by vitamin E supplementation. Kidney retinol concentrations were unaffected by MI or vitamin E supplementation.

Total retinyl esters are shown only for liver and kidney (Figure 3). Retinyl ester concentrations in plasma and myocardial tissue are not given because of their low values and high variability. Liver retinyl ester concentrations were not significantly different in the control and MI groups maintained on the basal diet. The vitamin E–enriched diet significantly increased retinyl esters in both control and MI groups. Retinyl ester concentrations significantly declined after MI in the kidneys of rats fed the basal diet compared with their sham controls, but these differences were not evident between the vitamin E–enriched, control, and MI groups.

Discussion

Antioxidants and Heart Failure: Cause and Effect

Previously, we reported that heart failure subsequent to MI in rats correlated with a decrease in antioxidant enzymes, catalase, and glutathione peroxidase, as well as an increase in

Figure 1. Vitamin E (Vit. E) in plasma (P), left ventricle (LV), right ventricle (RV), liver (L), and kidney (K) of rats fed a basal or vitamin E–enriched diet. All animals were subjected to either surgical MI or sham (control) procedures. Data are mean±SEM of 5 animals. Statistical differences (P<0.05) from sham controls of same diet treatment are denoted by *, and those between diet groups with the same surgical treatment are denoted by †. Note that the scale for concentration (vertical axis) given on the left is different for each tissue.

Figure 2. Effects of MI on retinol in plasma (P), left ventricle (LV), right ventricle (RV), liver (L), and kidney (K) of rats fed a basal or vitamin E (Vit. E)–enriched diet. All animals were subjected to surgery for either MI or sham control procedures. Data are mean±SEM of 5 animals. Statistical differences (P<0.05) from sham controls of same diet treatment are denoted by *, and those between diet groups with the same surgical treatment are denoted by †. Note that the vertical scale given on the left is different for each tissue.
Antioxidant Vitamins After MI

Oxidative stress. In the present study, depressed left ventricular function in the MI rats was also associated with a decrease in the nonenzymatic antioxidant vitamin E. There are now many experimental and clinical studies documenting a close relationship between antioxidant deficit and cardiac dysfunction. In the present study, a diet supplemented with vitamin E improved endogenous levels of this vitamin and modulated the decline in cardiac function in the MI group. These data clearly demonstrate that an increase in myocardial antioxidant concentrations helps sustain cardiac function subsequent to MI, thus suggesting that a decrease in antioxidant reserve may be a causative factor.

Antioxidant Dynamics: Tissue and Plasma Concentrations

The heart contains a relatively small fraction of the total antioxidant vitamin complement in the body. Any depletion of vitamins (E and A) in the heart tissue or plasma may be buffered by mobilization of these vitamins from the other large storage pools. How vitamins E and A are absorbed in the gut and reach the heart, liver, and kidney through the plasma is shown in Figure 4. Unlike vitamin A, vitamin E concentrations in the plasma are not tightly regulated by a specific transport protein, and they may fluctuate significantly. However, the liver still plays an important role in the distribution of vitamin E to extrahepatic tissues, including the heart. The present study highlights the importance of monitoring tissue samples to assess antioxidant vitamin content for the kidney. It is obvious that obtaining tissue samples to assess antioxidant vitamin content is not practical in a clinical setting. In this regard, at least 2 studies have recently shown that short-term peaks of vitamin A in the plasma, after an administered dose, can be used to calculate liver stores of the vitamin.

Vitamin E Changes

Although concentrations of vitamin E were not different in the plasma of rats subjected to MI, significant depletions of this vitamin did occur in the myocardium and liver. It has been reported that myocardial vitamin E concentrations decline in response to disruptive cardiac events, including MI. This decline probably reflects increased demand for the vitamin due to elevated oxidative stress. Although the percent decline in vitamin E in the left ventricle (32%) and liver (37%) of rats with MI in the present study was comparable, the liver, because of its larger size, suffered a much higher net loss of vitamin E. Taking tissue weight into account, the total loss of vitamin E in liver was 550 μg compared with a 15-μg loss from the heart. Possibly, the depletion of vitamin E from the liver occurs as the vitamin is mobilized from this large storage pool to replenish the depleted vitamin level in the oxidatively stressed left ventricle. Others have also noted the importance of the liver in redistributing vitamin E to tissues with transitory higher requirements.

In addition, our results show that by supplementing the diet with vitamin E, myocardial and hepatic concentrations of vitamin E can be elevated relative to baseline levels, even in animals with surgical MI. It is likely that MI does not influence vitamin E absorption at the gut level. Results from the kidney suggest that this organ is not as important as the liver for storing or supplying vitamin E to the heart after MI.

Vitamin A Changes

How the heart derives its retinol from the plasma and how both the liver and kidney can release retinol from the esterified forms when retinol binding protein (RBP) in the plasma is unoccupied by the retinol ligand are shown in Figure 3. In the current study, retinol concentrations in both the plasma and left ventricular tissue were unaffected by MI. However, liver retinol concentrations declined by 34% in rats with surgical MI compared with sham control rats, representing a mean total loss of ~60 μg of retinol after MI. This depletion occurred despite an ample supply of vitamin A from the commercial diet used in the present study (ie, 1160 IU/kg body weight per day). It has been reported that 50 000 IU/d of dietary vitamin A, which for a 75-kg person translates to 667 IU/d per kilogram, given as a part of an antioxidant therapy regimen, was effective in reducing oxidative stress and infarct size in patients with suspected MI. The relatively high supply in the basal diet in the present study may have contributed to the fact that storage forms of vitamin A, ie, total retinyl esters, were not significantly depleted in the liver of MI rats.

In contrast to the liver, total retinyl esters (ie, retinyl octanoate plus retinyl palmitate plus retinyl stearate) in the kidney declined significantly. The kidneys contribute a significant amount of retinol to the plasma by recapturing retinol and RBP that are not bound to transthyretin (TTR) and are therefore small enough to be filtered through the glomerulus. The recaptured retinol is either esterified or bound to RBP for reentry to the plasma pool. Actually, newly acquired retinol is preferentially esterified over resident intracellular retinol. Delivery of retinol by the complete plasma transport complex (retinol-RBP-TTR) appears to be important for this process, at least in liver stellate cells. Because kidneys maintain...
retinol concentrations by scavenging retinol through the glomerulus in a form that can be preferentially esterified by the kidney stellate cells, this process may be compromised after MI.

Supplementation with dietary vitamin E elevated plasma concentrations of vitamin A and allowed kidney vitamin A esters to return to baseline, even after MI. These effects are almost certainly related to the fact that vitamins E and A are complimentary antioxidants, such that supplementation with vitamin E spares vitamin A. Retinol has been shown to be an effective peroxyl radical scavenger. In fact, it may be even more effective than tocopherol, by virtue of its shorter polyene chain that affords it increased mobility and better access to peroxyl radicals in the membrane. However, the ability of retinol to scavenge aqueous initiator species is far below that of tocopherol owing to the fact that it lacks a hydroxyl group at the membrane surface. Rats supplemented with vitamin E still had lower vitamin A in the liver after MI, which suggests that this organ may experience different stresses on its vitamin A pool and its handling capabilities after MI.

Conclusions
Results from this study suggest that analysis of plasma as the sole indicator of vitamin status may not yield accurate information because this vector is affected much later in the evolution of heart failure, when storage-organ concentrations are depleted. Analyses of antioxidants in storage organs, therefore, offers a more reliable indication of antioxidant consumption after disruptive cardiac events. Furthermore, dietary supplements of vitamin E increase storage-organ concentrations of vitamins E and A and maintain cardiac homeostasis.
Antioxidant Vitamins After MI

function, even after MI. Although it is clear that storage-organ concentrations are an important part of antioxidant vitamin homeostasis, additional studies are required to elucidate their exact kinetic relationships.

Acknowledgments

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References


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