Cardioprotection with palm oil tocotrienols: comparison of different isomers

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Most of the health benefits of tocotrienols have been related to the immune response, cancer, and reproductive health (4, 17, 28). Recently, tocotrienols have been found to be effective in protecting the central nervous system compared with α-tocopherol, and tocotrienol inhibited glutamate-induced proteosomal c-Src kinase activation in HT4 neuronal cells (27). Tocotrienols have also been found to provide cardioprotection against several cardiovascular diseases (7, 23–25). A recent study showed that cardioprotection with palm tocotrienols (the tocotrienol-rich fraction [TRF]) was linked with their ability to stabilize proteasomes (7). We (21) have shown that the proteasome may become dysfunctional as a result of ischemia. TRF was observed to partially protect the proteasome, which may have facilitated the degradation of phospho-c-Src (7).

TRF of palm oil consists of three isomers of tocotrienols (α, γ, and δ) as well as α-tocopherol. Although these isomers possess comparable antioxidant properties, their abilities to potentiate signal transduction could be different. For example, protein tyrosine phosphorylation plays a central role in the execution of death. Nanomolar amounts of α-tocotrienol, but not α-tocopherol, blocked glutamate-induced death by suppressing glutamate-induced early activation of c-Src kinase (27). Activation of pp60 c-Src kinase and phosphorylation of ERK were observed in response to glutamate treatment. Overexpression of kinase-active c-Src sensitized cells to glutamate-induced death. Tocotrienol treatment prevented the death of Src-overexpressing cells treated with glutamate. α-Tocotrienol did not influence the activity of recombinant c-Src kinase, suggesting that its mechanism of action may include the regulation of Src homology domains.

To determine which isomers of tocotrienol were responsible for the preservation of proteasomes, rats were gavaged α-, γ-, or δ-isomers of tocotrienol, and the results were compared with those of TRF. The results of our study showed that all three isomers of tocotrienol could stabilize the proteasome, with the δ-isomer completely preventing dysfunction and the γ- and α-isomers resulting in partial protection. However, to our surprise, the beneficial effects of the isomers on postischemic function, Akt, and c-Src appear to be inversely related to the degree of protection of the proteasome, suggesting that this degradative organelle may help to maintain a balance between cell death and survival signals and that some dysfunction might be necessary to facilitate the generation of survival signals.

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MATERIALS AND METHODS

Chemicals. Various isomers of tocotrienols (97% pure) were obtained from Carotech (BASF, Florham Park, NJ). TRF was a generous gift from the Malaysia Palm Oil Board. TRF and α-, γ-, and δ-tocotrienols were solubilized in 0.01% ethanol, which served as the vehicle control. Three different concentration of TRF (0.35%, 1%, and 3.5%) were given to rats by gavage for two different periods of time (2 and 4 wk) to determine the optimal dose and time of feeding. All other chemicals were of analytical grade and were obtained from Sigma Chemical (St. Louis, MO) unless otherwise specified.

Animals. Male Sprague-Dawley rats (250 –275 g body wt) were used in this study. All animals used in this study received humane care in compliance with the principles of the laboratory animal care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals, prepared by the National Academy of Sciences and published by the National Institutes of Health (Publication No. 85–23, Revised 1996). The animal protocol was approved by the Animal Care Committee of the University of Connecticut Health Center. They were provided with food and water ad libitum all the time until the start of the isolated heart preparation. Rats were randomly assigned to one of the following groups: control; 0.01% ethanol; 0.35%, 1%, or 3.5% TRF, or 0.03, 0.3, or 3 mg/kg body wt of α-, γ-, or δ-tocotrienol. Rats were gavaged (forcefully injected into the stomach by using a stomach needle (1.2 mm diameter) every day for either 2 or 4 wk for TRF treatment and only 4 wk for the individual isoform treatments) with 0.5 ml of either 0.01% ethanol (control) or one of the above-mentioned experimental compounds. Before performing our experiments with 0.3 mg/kg body wt of the three different types of tocotrienol, we determined the optimal dose of these isoforms by studying three different doses (0.03, 0.3, and 3 mg/kg body wt). In the 0.03 mg/kg body wt groups, none of the isoforms had any cardioprotective effects, and for the 3 mg/kg body wt groups, observations were very similar to the 0.3 mg/kg body wt groups (data not shown). There were ~200 μl of all three pure isoforms of tocotrienol present in the 0.3 mg/kg body wt dose.

Isolated working rat heart model. Rats were anesthetized with pentobarbital sodium (80 mg/kg ip, Abbott Laboratories, North Chicago, IL), and heparin sodium (500 IU/kg iv, Elkins-Sinn, Cherry Hill, NJ) was administered for anticoagulation. After a sufficient depth of anesthesia had been ensured, a thoracotomy was performed, and hearts were perfused in the retrograde Langendorff mode at 37°C at a constant perfusion pressure of 100 cmH2O (10 kPa) for a 5-min washout period (6). The perfusion buffer consisted of a modified Krebs-Henseleit buffer [containing (in mM) 118 NaCl, 4.7 KCl, 1.7 CaCl2, 25 NaHCO3, 1.2 KH2PO4, 1.2 MgSO4, and 10 glucose]. The Langendorff preparation was switched to the working mode after the washout period, as previously described (8). The working mode was introduced by switching the flow to the left atrium from the aortic root with a constant preload of 17 cmH2O and an afterload of 100 cmH2O.

After 10 min, when cardiac function had attained a steady state, baseline functional parameters were recorded. Hearts were then subjected to global ischemia for 30 min followed by 2 h of reperfusion. Reperfusion was in the retrograde mode for the first 10 min to allow for postischemic stabilization and thereafter in the antegrade working mode to allow for the assessment of functional parameters, which were recorded at 10, 30, 60, and 120 min of reperfusion.

Cardiac function assessment. Aortic pressure was measured using a pressure transducer (model P23XL, Gould Instrument Systems, Valley View, OH) connected to a sidearm of the aortic cannula; the signal was amplified using a signal conditioner (series 6600, Gould) and monitored on a real-time data acquisition and analysis system (CORDAT II, Triton Technologies, San Diego, CA) (8). Heart rate,
left ventricular developed pressure (LVDP; defined as the difference between maximum systolic and diastolic aortic pressures), and the first derivative of developed pressure (dP/dt) were derived or calculated from the continuously obtained pressure signal (6). Aortic flow (AF) was measured using a calibrated flowmeter (Gilmont Instruments, Barrington, IL), and coronary flow was measured by a timed collection of the coronary effluent dripping from the heart.

**Infarct size estimation.** At the end of reperfusion, a 10% (wt/vol) solution of triphenyltetrazolium chloride in phosphate buffer was infused into the aortic cannula for 20 min at 37°C (3). Hearts were excised and stored at −70°C. Sections (0.8 mm) of both ventricles of the frozen heart were fixed in 2% paraformaldehyde, placed between two coverslips, and digitally imaged using a scan maker (model 600z, Microtek). We used all three fragments of the heart and calculated the mean value. To quantitate the areas of interest in pixels, NIH Image 5.1 (a public domain software package) was used. The infarct size was quantified and expressed in pixels.

**Evaluation of apoptosis.** Immunohistochemical detection of apoptotic cells was carried out using TUNEL (5) using a TUNEL kit (Oncor, Gaithersburg, MD). Heart tissues were immediately put in 10% formalin and fixed in an automatic tissue fixing machine. Tissues were carefully embedded in molten paraﬃn in metallic blocks, covered with ﬂexible plastic molds, and kept under freezing plates to allow the paraﬃn to solidify. The metallic containers were removed, and tissues became embedded in paraﬃn on the plastic molds. Prior to tissues were analyzed for apoptosis, sections were deparaffinized with xylene and washed in succeesion with different concentrations of ethanol (absolute, 95%, and 70%). Tissues were then incubated with mouse monoclonal antibody recognizing cardiac myosin heavy chain to speciﬁcally recognize apoptotic cardiomyocytes. Fluorescence staining was viewed with a confocal laser microscope. The number of apoptotic cells was counted and expressed as a percentage of the total myocyte population.

**Estimation of malondialdehyde.** Malondialdehyde (MDA) was assayed in the heart as previously described (2) to monitor the development of oxidative stress. MDA was derivatized using 2,4-dinitrophenylhydrazine. Aliquots of 25 μl derivatized MDA in acetonitrile were injected into a Beckman Ultrasphere C18 (3 mm) column in a Waters (Milford, MA) HPLC. The products were eluted isocratically and detected at 307, 325, and 356 nm. The amount of MDA was quantitated using Maxima software program (Waters).

**Western blot analysis.** Heart tissues were homogenized and suspended (5 mg/ml) in sample buffer [10 mM HEPES (pH 7.3), 11.5% sucrose, 1 mM EDTA, 1 mM EGTA, 1 mM diisopropyl fluorophosphate, 0.7 mg/ml pepstatin A, 10 mg/ml leupeptin, and 2 mg/ml aprotinin]. Proteins were then solubilized with the addition of the same amount of 2× Laemmli solution [9% (wt/vol) SDS, 6% (vol/vol) β-mercaptoethanol, 1% (vol/vol) glycerol, and a trace amount of bromophenol blue dye in 0.05 M Tris-HCl (pH 6.7)]. The cellular proteins (50-μl samples) were electrophoresed using 10% SDS-PAGE and then transferred to Immobilon-P membranes (Millipore) using semidiﬀusion transfer system (Bio-Rad). Proteins were then solubilized (5 mg/ml) in sample buffer [10 mM HEPES (pH 7.3), 11.5% sucrose, 1 mM EDTA, 1 mM EGTA, 1 mM diisopropyl fluorophosphate, 0.7 mg/ml pepstatin A, 10 mg/ml leupeptin, and 2 mg/ml aprotinin]. Proteins were then solubilized with the addition of the same amount of 2× Laemmli solution [9% (wt/vol) SDS, 6% (vol/vol) β-mercaptoethanol, 1% (vol/vol) glycerol, and a trace amount of bromophenol blue dye in 0.05 M Tris-HCl (pH 6.7)]. The cellular proteins (50-μl samples) were electrophoresed using 10% SDS-PAGE and then transferred to Immobilon-P membranes (Millipore) using a semidiffusion transfer system (Bio-Rad). Immunoblots were blocked in Tris-buffered saline-Tween 20 [0.2% (vol/vol) Tris base (pH 7.6), 137 mM NaCl, and 0.1% Tween 20] supplemented with 5% BSA for 1 h, incubated with 1:100 diluted primary rabbit antibodies speciﬁcally against Akt, phospho-Akt, Src, phospho-Src (Santa Cruz biotechnology) for 4 h, and then incubated with 1:10,000 diluted secondary antibodies of horseradish peroxidase-conjugated anti-rabbit IgG (Boehringer Mannheim) for 1 h at room temperature. After three washes of 5 min each, blots were treated with enhanced chemiluminescence (Amersham) reagents, and bands of Src and Akt (both phosphorylated and nonphosphorylated) were detected by autoradiography for variable lengths of time (15 s to 3 min) with Kodak X-Omat film (16).

**Protective activity.** ATP- and non-ATP-dependent activities of the proteins were determined using the method of Grune et al. (11).
as modified by Powell et al. (20) in the presence of ATP ranging from 0 to 56 μmol/l. The specificity of the assay was confirmed by the inclusion of lactacystin (10 μmol/l) in a subset of the reactions.

Statistical analysis. Values for myocardial functional parameters, total and infarct volumes, and infarct sizes are expressed as means ± SE. ANOVA was followed by Bonferroni’s correction to test for any differences between mean values of all groups. If differences between groups were established, values of each treated group were compared with those of the control group by a modified t-test. Proteasome results were analyzed using one-way ANOVA followed by Tukey’s post hoc analysis. Results were considered significant if P < 0.05.

RESULTS

Effects of TRF and α-, γ-, and δ-tocotrienol on ventricular function. Initially, we performed dose-response experiments to determine the optimal dose with the effective duration of TRF treatment. There were no effects on ventricular function at 0.35% or 1% TRF for either 2 or 4 wk as well as no significant effects with 3.5% TRF fed for 2 wk (Figs. 1 and 2). The maximum beneficial effect was noticed with 3.5% TRF gavaged for 4 wk.

Similar to TRF, for tocotrienols, we performed dose-response experiments to determine the optimal dose of all three different isoforms of tocotrienol (α, γ, and δ) present in TRF. Since TRF did not exert any cardioprotective effect after 2 wk, we chose to gavage tocotrienols only for 4 wk. At a dose of 0.03 mg/kg body wt for 4 wk, none of the isoforms had any significant protective effects, and at the dose of 3 mg/kg body wt, both the functional parameters (data not shown) and infarct size (see Fig. 5B) were very similar to the 0.3 mg/kg body wt dose (Figs. 3, 4 and 5B). Thus, the results demonstrated the optimal dose of tocotrienol isomers to be 0.3 mg/kg body wt given for 4 wk.

There were no differences in baseline function among any of the groups in either set of experiments. In general, there were no significant differences between the TRF-treated and control groups or between α-, γ-, and δ- isomers in heart rate (data not shown; heart rate was 305 ± 15 beats/min before ischemia and 285 ± 12 beats/min after 2 h of reperfusion, which was not statistically significant) and coronary flow (Figs. 2B and 4B). Upon reperfusion, absolute values of all functional parameters were decreased in all groups compared with respective baseline values. The TRF-treated group displayed a significant recovery of postischemic myocardial function. AF was markedly higher in the 3.5% TRF-treated group from 30 min of reperfusion onward (Fig. 2A). The cardioprotective effects of TRF were evidenced by significant differences in LVDP from 30 min of reperfusion onward. A similar kind of difference was found also in dP/dt compared with TRF-treated animals (Fig. 1, A and B). Like TRF, the postischemic ventricular recovery with all three isoforms of tocotrienol was better than the vehicle control but not significant except for γ-tocotrienol and, in some cases, α-tocotrienol (Figs. 3 and 4). Maximum cardioprotection was noticed for γ-tocotrienol followed by α-tocotrienol. In contrast, there were no significant differences with δ-tocotrienol compared with the control for any cardiac function. The cardioprotective effect of γ-tocotrienol was significantly greater, as documented by higher AF, AF,

![Fig. 3. Effects of three different isoforms (α-, γ-, and δ-) of tocotrienol on ventricular performance of isolated hearts. Rats were fed (by gavage) α-, γ-, and δ-tocotrienol (0.3 mg/kg body wt) for 4 wk. At the end of 4 wk, isolated hearts were prepared, and hearts were made globally ischemic for 30 min followed by 2 h of reperfusion in the working mode. LVDP (A) and LV dP/dt (B) were determined at the indicated times. Results are expressed as means ± SE of 6 hearts/group. *P < 0.05 vs. control.](http://ajpheart.physiology.org/)
LVDP, and dP/dt. AF was significantly higher from 60 min of reperfusion onward compared with vehicle-treated animals (Fig. 4A). α-Tocotrienol showed a significant increase only at 120 min of reperfusion (Fig. 4A). Similarly, both LVDP and LV dP/dt increased only with β-tocotrienol (Fig. 3, A and B).

**Effects of TRF and tocotrienols on myocardial infarct size.** TRF (3.5%) fed for 4 wk showed the maximum reduction of infarct size compared with the control group (24.7 ± 2.23% vs. 34.66 ± 1.87%; Fig. 5A). All three isomers of tocotrienol failed to improve myocardial infarct size with the 0.03 mg/kg body wt dose for 4 wk compared with the vehicle-treated group. Consistent with the results of ventricular function, the 3 mg/kg body wt dose of all three isomers of tocotrienol given for 4 wk also showed a similar pattern of protection as found for the 0.3 mg/kg body wt dose for 4 wk of treatment (Fig. 5B).

Again, γ-tocotrienol showed the highest cardioprotective effect among the three isomers by reducing the infarct size by 22.4 ± 2.11 compared with control. α-Tocotrienol also reduced the infarct size significantly, whereas there were no differences with δ-tocotrienol (Fig. 6A).

**Effects of palm-tocotrienols on cardiomyocyte apoptosis.** The percentage of apoptotic cardiomyocytes was reduced with all the isoforms of tocotrienol. The reduction was the highest with the 0.3 mg/kg body wt dose of γ-tocotrienol treatment for 4 wk compared with the vehicle-treated group (Fig. 6B). α-Tocotrienol also reduced the number of apoptotic cardiomyocytes significantly (345 ± 35 vs. 467 ± 50 cardiomyocytes/100-unit area; Fig. 6B).

**Effects of palm-tocotrienols on proteasome activities.** Several parameters of proteasome function were assessed by measuring the cleavage of substrate in the absence and presence of an optimal concentration of ATP that maximally stimulates activity (20) (Table 1). Neither ischemia-reperfusion nor the isomers had an effect on non-ATP-dependent activity, which is reflective of the activity of the core 20S proteasome catalytic subunit. Total proteasome activity, reflective of the activity of all forms of the proteasome, was diminished by ischemia-reperfusion, an effect that was completely prevented by the δ-isomer but only partially prevented by the α- and γ-isomers. Accordingly, ATP-dependent activity, reflective of the function of the 19S regulatory subunit, was decreased to less than half by ischemia-reperfusion, completely protected by the δ-isomer, and only partially protected by the α- and γ-isomers. Due to a paucity of samples for proteasome determination, no statistical analysis was possible.

**Effects of palm-tocotrienols on MDA formation in the heart.** MDA is the presumptive marker for lipid peroxidation, which, in turn, is the marker for the development of oxidative stress and ROS activity in the heart. As shown in Fig. 7, the MDA content was significantly less in hearts of the animals fed either α-, γ-, or δ-tocotrienol at all reperfusion time points. The most promising observation was with γ-tocotrienol, where the reduction was the highest among the three isoforms at all three reperfusion time points compared with the vehicle-treated group (0-min reperfusion: 2.19 vs. 6.11 ng/ml, 10-min reperfusion: 2.45 vs. 5.27 ng/ml, and 30-min reperfusion: 2.02 vs. 4.49 ng/ml).

**Effects of palm-tocotrienols on c-Src and Akt expression and phosphorylation.** Compared with the ischemia-reperfusion group, treatment with all three tocotrienol isoforms (α, γ, and
δ) inhibited the expression of phosphorylated c-Src, but only γ-tocotrienol induced a significant reduction. Treatment with γ- and δ-tocotrienol (Fig. 8) resulted in a significant enhancement of Akt phosphorylation compared with the ischemia-reperfusion group (Fig. 8). In contrast, there were no significant increases in Akt phosphorylation with δ-tocotrienol compared with ischemia-reperfusion.

DISCUSSION

The present study demonstrates that TRF fed (gavaged) for a period of 4 wk provided cardioprotection, as evidenced by improved ventricular function and reduced myocardial infarct size and cardiomyocyte apoptosis. TRF contains significant amounts of three isoforms of tocotrienol (α: 22.1%, γ: 45.7%, and δ: 10.4%). Studies have shown that, even in nanomolar concentrations, tocotrienols provide cellular protection. The

![Fig. 5. Dose-response results of the effects of TRF and three different isoforms of tocotrienol (α, γ, and δ) on myocardial infarction.](image)

![Fig. 6. Effects of three different isoforms of tocotrienol (α, γ, and δ) on the ischemia-reperfused (I/R) heart.](image)

![Table 1. Proteasome activities in control and tocotrienol-treated rat hearts](table)

Values for proteasome activity represent changes in fluorescence over 30 min and are expressed as means of 2 determinations in each group. The different proteasome activities are defined as follows: non-ATP-dependent activity, activity in the absence of ATP; total, maximal activity in the presence of the optimal ATP concentration; ATP-dependent activity, total – non-ATP-dependent activity; and activation, total/non-ATP-dependent activity.

**Table 1.** Proteasome activities in control and tocotrienol-treated rat hearts

<table>
<thead>
<tr>
<th>Group</th>
<th>Non-ATP Dependent</th>
<th>Total</th>
<th>ATP Dependent</th>
<th>Activation, times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>63</td>
<td>152</td>
<td>89</td>
<td>2.47</td>
</tr>
<tr>
<td>I/R</td>
<td>58</td>
<td>99</td>
<td>41</td>
<td>1.75</td>
</tr>
<tr>
<td>α-Tocotrienol</td>
<td>52</td>
<td>114</td>
<td>62</td>
<td>2.17</td>
</tr>
<tr>
<td>γ-Tocotrienol</td>
<td>60</td>
<td>123</td>
<td>63</td>
<td>2.05</td>
</tr>
<tr>
<td>δ-Tocotrienol</td>
<td>73</td>
<td>159</td>
<td>86</td>
<td>2.18</td>
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bioavailability of α-, γ-, and δ-tocotrienols under the fed state over those of the fasted state have been found to lie between 2.24 – 3.40%, 2.05 – 4.09%, and 1.59 – 3.81%, respectively (4). To determine the cardioprotective role of the different isomers of tocotrienol, we simultaneously gavaged rats with pure α-, γ-, and δ-tocotrienol and found that γ-tocotrienol and, to some extent, α-tocotrienol, but not δ-tocotrienol, were cardioprotective (the degree of cardioprotection was greater for γ-tocotrienol than for α-tocotrienol). In each case, the optimal dose was used. This was determined from dose- and time-response curves. The examination of survival signals by TRF and tocotrienols revealed that these compounds inhibited the activation of c-Src and induced the phosphorylation of Akt, suggesting the participation of the phosphatidylinositol 3-kinase-Akt survival pathway in cardioprotection.

A recent study (7) from our laboratory has shown that in acute experiments, when isolated rat hearts were pretreated with TRF, it reduced myocardial ischemia-reperfusion injury through stabilization of the proteasome. This study also showed activation of c-Src during ischemia and reperfusion, and TRF prevented such activation of c-Src. The present study, using a chronic experimental setup where animals were fed TRF and tocotrienols for a period of 4 wk, confirmed this previous observation and further demonstrated that tocotrienol isomers present in TRF differentially stabilize proteasomes with the δ-isomer being the most protective and α- and γ-isomers providing protection midway between baseline and post-ischemic hearts, which surprisingly is reverse to their respective cardioprotective efficacies. It is not clear whether this is related to the differential regulation of proteasome subunits, but it could be related to differential compartmentalization (30) or antioxidant potential, with the δ-isomer reported to be the most effective (19).

Vitamin E, commonly known as α-tocopherol, represents a family of four tocopherols and four tocotrienols, with the four isoforms being designated as α, β, γ, and δ, based on the number of methyl groups on the chromanol ring. Tocotrienols differ from the corresponding tocopherols only in their aliphatic tail. The isoforms of tocotrienols differ in their methyl substitution in the chromanol head and a 16-carbon hydrocarbon tail; the α-isofrom contains three methyl groups, whereas the β- and γ-isomers have two methyl groups, and the δ-isofrom has only one methyl group (30). Tocotrienols have three double bonds and an unsaturated side chain that renders them greater fluidity and makes them easily accessible to cells with saturated fatty acid layers to be incorporated into cell membranes.

A growing body of evidence supports the notion that tocotrienols could be superior to tocopherols as far as the health benefits are concerned. Although some studies have shown...
higher antioxidant properties of tocotrienols over tocopherols for superiority, several other studies, including the present study, have indicated tocotrienols as signaling molecules that protect the heart from cellular injury (27). As mentioned earlier, only a handful of studies have been performed with tocotrienols compared with those with α-tocopherol. Most of the studies performed to date with tocotrienols have indicated their potent anticancer effects (15). Tocotrienols, especially δ-tocotrienol, have been found to be very effective in promoting apoptosis and killing breast cancer cells (31). In contrast, γ-tocotrienol was more effective compared with the chemotherapy drug tamoxifen in inhibiting the growth of cultured human breast cancer cells (12). Tocotrienols from TRF inhibited the proliferation of human breast cancer cell lines (18). Tocotrienols also suppressed the growth of murine B16 melanomas in vitro and in vivo (15).

Only recently, other health benefits of tocotrienols have become apparent. For example, tocotrienols inhibited glucosmate-induced pp60 c-Src kinase activation of HT4 neuronal cells (27). TRF of rice bran dose-dependently reduced 12% of serum cholesterol in hypercholesterolemic humans (25). In another study, a 4-wk dietary supplement with δ-tocotrienol elicited a 5% decrease in the cholesterol levels of 36 human subjects (22). Subjects continuing on the dietary regimen for a second 4-wk period experienced an additional 2% decrease in their cholesterol levels. Supplementation with dietary tocotrienols from TRF of palm oil reduced the concentration of plasma cholesterol and apolipoprotein B, thromboxane B2, and platelet factor 4, indicating its ability to protect against endothelial dysfunction and platelet aggregation (23). Supplementation with TRF reduced plasma cholesterol levels in a human pilot study (24). Finally, our own study showed cardioprotection with TRF through its ability to stabilize proteasomes.

In summary, the cardioprotection mediated by tocotrienols does not appear to be potentiated by their antioxidant properties, as the isomers possess comparable antioxidant abilities. It is likely that the different degrees of cardioprotection by different isomers of tocotrienols are due to their differential effects of c-Src inhibition and/or proteasome stabilization, as observed in our study. Indeed, δ-tocotrienol was the most effective in inhibiting Src phosphorylation, which was also reflected in its ability to phosphorylate Akt, which constitutes a survival signal. Similar to our previous findings, the present study clearly demonstrated a role of the proteasome in tocotrienol-mediated cardioprotection. However, surprisingly, the cardioprotective effects were associated with the isomers that have the least protective effects on the proteasome. Clues as to why this might occur can be gained from studies of c-Src and Akt. One way of regulating Src kinase and active Src is through ubiquitin-mediated degradation (13, 14). Under conditions of proteasomal dysfunction, such as during ischemia, active c-Src would be expected to be increased, such as observed in this study. If not relieved, sustained increases in active Src are detrimental to the cell and are associated with prodeath signaling. By protecting the proteasome and maintaining some degree of function, the removal of active Src is facilitated. On the other hand, increases in Akt phosphorylation are generally associated with cardiomyocyte survival. Akt has recently been suggested to be subject to ubiquitin-mediated degradation (26, 29); thus, proteasomal dysfunction in this case would tend to result in the accumulation and generation of a prosurvival signal. In reality, whether a cell survives or not depends on the balance between cell death and cell survival signals. In the case of both the α- and γ-isomers, there is a maintenance of sufficient proteasomal function to facilitate the removal of active Src and yet enough dysfunction to still allow an accumulation of Akt. However, δ-tocotrienol treatment completely prevented proteasome dysfunction and also resulted in the lowest level of phosphorylated Akt, suggesting that at critical times, a certain amount of proteasomal dysfunction may be necessary to facilitate the propagation of survival signals. The cardiac hypertrophy literature provides support for this interpretation as activation of the proteasome is associated with maladaptive hypertrophy secondary to pressure overload (9). It is also worth mentioning that compared with α-tocopherol (4), tocotrienols have much higher cardioprotective properties.

REFERENCES


