Modulation by dietary vitamin E and selenium of clotting whole blood thromboxane A_2 and aortic prostacyclin synthesis in rats

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The effect of dietary vitamin E and selenium (Se) on aortic prostacyclin (PGI_2) and clotting whole blood thromboxane (TX) A_2 synthesis in 1-month-old Fischer 344 rats was investigated. Rats were fed basal semi-purified diets deficient in vitamin E and Se or basal diet supplemented with either 200 IU vitamin E and/or 0.2 ppm Se per kg diet for 2 months. Ex vivo production of TXB_2 and 6-keto-$PGF_{1\alpha}$, the corresponding stable metabolites of TXA_2 and PGI_2, were measured in whole blood and aortic rings, respectively. Animals fed vitamin E-deficient diet had significantly ($P < 0.05$) lower plasma levels of α-tocopherol than those fed vitamin E-supplemented diet. Plasma Se was undetected and activity of plasma glutathione peroxidase was diminished in rats fed Se-deficient diets. Vitamin E and Se treatments affected TXA_2 and PGI_2 production differently. Aortic PGI_2 synthesis was affected by both dietary vitamin E and Se, whereas whole blood TXA_2 synthesis was only affected by dietary vitamin E. These results suggest that vitamin E and Se have specific and different effects on TXA_2 and PGI_2 synthesis, and that their combined supplementation may have a favorable effect on PGI_2:TXA_2 ratio.

Keywords: vitamin E; selenium; prostacyclin; thromboxane; rat

Introduction

Prostacyclin (PGI_2) and thromboxane (TX) A_2 are arachidonic acid metabolites involved in controlling platelet activity and vascular tone. PGI_2, synthesized by both endothelial and vascular smoother muscle cells, is a potent vasodilator and endogenous inhibitor of platelet aggregation.[1,2] Conversely, TXA_2, produced mainly by blood platelets, acts as a vasoconstrictor and promotor of platelet aggregation.[3] The balance between arterial wall PGI_2 production and platelet TXA_2 has been suggested as an important factor in platelet activity and aggregation and may affect the incidence of arrhythmias in myocardial ischemia.[4] The importance of cellular peroxide tone in prostaglandin (PG) and leukotriene synthesis is well documented.[5] Lipid hydroperoxides at low levels are necessary for the activation of PGH synthetase; however, high levels can inhibit cyclooxygenase and thromboxane synthetase.[5]

Vitamin E scavenges free radicals and has a profound effect on platelet aggregation.[6-8] Selenium (Se)-dependent glutathione peroxidase (GSH-Px), a cytosolic antioxidant defense enzyme, catalyzes the breakdown of lipid hydroperoxides and hydrogen peroxide to less reactive alcohols and water, respectively. Thus, vitamin E and Se nutriture may have effects on the maintenance of aortic and platelet interactions that maintain blood homeostasis and may influence the cardiovascular consequences of impaired homeostasis.[8,9-11] The objective of this study was to elucidate the interaction of dietary vitamin E and Se on PGI_2 and TXA_2 formation.

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Methods and materials

Animals and diets

Twenty 1-month-old male Fischer 344 rats (Charles River Breeding Lab., Inc., Wilmington, MA USA) were randomly assigned to four dietary treatment groups. Group 1 (+E +Se) received the basal diet deficient in vitamin E and Se[12] supplemented with 200 IU vitamin E as α-tocopheryl acetate/Kg diet and 0.2 ppm Se as sodium selenite. Group 2 (+E −Se) received the basal diet supplemented only with 200 IU vitamin E/Kg diet. Group 3 (−E +Se) received the basal diet supplemented only with 0.2 ppm Se. Group 4 (−E −Se) received only the basal diet. Food and water was provided ad libitum. Rats were housed individually in stainless-steel wire mesh cages and were maintained at 22° C with 12-hr light-dark cycles.

Tissue preparation and incubation

After 8 wks of dietary treatment, animals were fasted overnight then sacrificed by decapitation. Blood was collected and serum separated and stored at −70° C for vitamin E and Se analysis. Aorta was removed rapidly and rinsed with 0.1 M potassium phosphate buffer (Kpi), pH 7.4. After removal of adherent fatty tissue, aortas were sectioned in rings approximately 2 mm thick, weighed, and incubated in 1.0 mL of 0.1 M Kpi buffer at 37° C in a shaking water bath for 30 min. Incubation medium was then removed for determination of 6-keto-$PGF_{1\alpha}$ a stable metabolite of PGI_2. Incubation of aorta for 30 min has been demonstrated as adequate to produce an effect of vitamin E on PGI_2.[13] One mL of clotting whole blood was incubated at 37° C in a shaking water bath for 10 min at which time 0.1 mL of 42 mmol/L aspirin solution was added to inhibit cyclooxygenase enzyme activity. Following serum separation by centrifugation at 4° C for 10 min, TXB_2 was measured by RIA. TXB_2 levels in sera separated from blood samples at 37° C were shown to correlate significantly ($P < 0.001$) with the TXB_2 production in platelet-rich plasma, indicating that this method can be used to assess platelet synthesis of TXB_2.[14]

Biochemical measurements

6-keto-$PGF_{1\alpha}$ in medium and TXB_2 a stable metabolite of TXA_2 in serum were measured by RIA.[15] The details of antibody specificity and cross reactivity have been published.[15] PG standards were a gift from Upjohn Company (Kalamazoo, MI USA) and tritiated standards were purchased from New England Nuclear (Boston, MA USA). The antibodies were a gift from Drs. J. Dupont and M. Mathais of Colorado State University (presently at the United States Department of Agriculture, Washington, DC USA). Activity of GSH-Px in plasma was assayed by method of Paglia and Valentine,[16] and the level of Se in plasma was determined by Perkin Elmer 5000 Zeeman furnace (Norwalk, CT USA) atomic absorption spectrophotometry.[17] Plasma α-tocopherol was measured by a high performance liquid chromatography method.[18]

Statistical analysis

Data were analyzed using a VAX-11/780 computer (Digital Equipment Co., Maynard, MA USA) and Statistical System Software (SAS Institute, Cary, NC USA). Using general linear models procedures, factorial analysis of variance was performed for the overall effect of dietary treatments on the variables measured. Difference of means were evaluated for significance by multiple means comparison test.

Results

Dietary treatments had no significant effect on the overall weight gain of animals for the test period. The vitamin E-deficient diets significantly ($P < 0.05$) reduced plasma α-tocopherol with 3–11% of the level observed in vitamin E-supplemented groups, and the Se-deficient diets reduced Se to undetectable levels in plasma (*Table 1*). Analysis of variance showed no significant interaction between vitamin E and Se on the level of plasma vitamin E or Se. However, a significant interaction between vitamin E and Se was observed on plasma activity of GSH-Px. The highest activity of plasma GSH-Px was observed in rats receiving 200 IU vitamin E and 0.2 ppm Se in their diet (group 1). Although rats in group 3 (−E +Se) received 0.2 ppm Se in their diet, vitamin E deficiency significantly ($P < 0.05$) lowered the GSH-Px activity of plasma in these rats when compared with group 1 (+E +Se).

Analysis of variance indicated an overall significant effect of diet on TXA_2 ($P = 0.005$) and on PGI_2 ($P = 0.055$) synthesis. Aortic rings obtained from rats supplemented with 200 IU of dietary vitamin E and 0.2 ppm Se (group 1) synthesized a higher level of PGI_2 ($P < 0.05$) than any other group (*Table 2*). Ex vivo synthesis of TXA_2 in clotting whole blood from rats fed the vitamin E-deficient diets (groups 3 and 4) was significantly ($P < 0.01$) higher than those fed vitamin E-supplemented diets (groups 1 and 2).

Discussion

Regulation of blood and cardiovascular homeostasis in normal and pathological conditions is influenced by eicosanoid production. Pharmacological modulation of eicosanoid synthesis has been used to alter platelet aggregation, inhibit post-operative thrombosis, and reduce cardiovascular risk factors.[19,20]

Dietary vitamin E and Se have been implicated in regulation of the enzymatic oxygenation of the arachidonic acid cascade. The effect of vitamin E on the arachidonic acid cascade may be mediated by modulation of phospholipase A_2 activity[17,21,22] or by alteration in cyclooxygenase or lipooxygenase activities.[10] The effect of Se on arachidonic acid metabolism appears to occur through the antioxidant function of GSH-Px in modulating phospholipase A_2 activity, by controlling lipid hydroperoxide production, and/or altering the activities of lipooxygenase and cyclooxygenase.[5] The presence of lipid hydroperoxides in low levels are necessary for the activation of PGH synthase. However, an over-abundance of these products can result in the activation of cyclooxygenase while increasing TXA_2 synthesis.[5]

The present study demonstrates the modulatory effect of dietary vitamin E and Se on prostanoid synthesis, as evident from altered ex vivo synthesis of aortic PGI_2 and platelet TXA_2 production. Antioxidants can modulate prostanoid synthesis by affecting the level of fatty acid hydroperoxides, but the sensitivity and regulation of thromboxane synthetase to peroxide tone may be

Table 1 Concentration of vitamin E and selenium and activity of glutathione peroxidase in plasma

Group number	Dietary treatment	α-tocopherol μg/dL	Selenium μg/dL	GSH-Px Activity U/mL
1	+E+Se	722 ± 4[a]	0.65 ± 0.24[a]	13.04 ± 0.24[a]
2	+E−Se	735 ± 50[a]	ND	0.15 ± 0.24[c]
3	−E+Se	24 ± 8[b]	0.51 ± 0.07[a]	10.80 ± 0.37[b]
4	−E−Se	82 ± 33[b]	ND	0.24 ± 0.09[c]

Values are means ± SEM; means not sharing a common letter superscript are significantly different ($P < 0.05$).
ND, not detectable.
+E = 200 IU α-tocopheryl acetate/kg diet; −E = no vitamin E added into diet; +Se = 0.2 ppm selenium in the diet; −Se = no selenium added.

Table 2 Ex vivo synthesis of prostacyclin by aortic rings and thromboxane A_2 by whole blood

Group number	Dietary treatment	6-Keto $PGF_{1\alpha}$[a] μg/gm	TXB_2[a] ng/mL
1	+E+Se	8.17 ± 1.10[a*]	226 ± 30[a**]
2	+E−Se	3.84 ± 0.90[b]	230 ± 20[a**]
3	−E+Se	4.38 ± 0.91[b]	410 ± 20[b]
4	−E−Se	4.64 ± 0.76[b]	400 ± 40[b]

Values are means ± SEM; means not sharing a common letter superscript are significantly different ($^*P < 0.05$ and $^{**}P < 0.01$).
+E = 200 IU α-tocopheryl acetate/Kg diet; −E = no vitamin E added to diet; +Se = 0.2 ppm selenium in the diet; −Se = no selenium added.
[a]6-keto $PGF_{1\alpha}$ and TXB_2 are the stable metabolites of prostacyclin (PGI_2) and thromboxane A_2, respectively.

different and independent from that of prostaglandin synthetase.[8]

Although tissue vitamin E and GSH-Px levels were not measured here, earlier reports from this and other laboratories demonstrate that plasma levels of these antioxidants are reflective of diet-induced changes in tissue levels of rats.[23,24] Ex vivo production of TXA_2 by clotting whole blood was affected neither by supplementation nor deficiency of Se. In contrast, vitamin E deficiency increased formation of TXA_2 by clotting whole blood. This effect was evident when vitamin E-deficient rats (groups 3 and 4) were compared with rats fed diets containing 200 IU vitamin E (groups 1 and 2). Hwang and Donovan[25] reported that synthesis of TXB_2 in diluted whole blood stimulated by collagen was significantly lower in vitamin E-supplemented rats compared with vitamin E-deficient rats. The increased TXA_2 formation in clotting whole blood from vitamin E-deficient rats in part can be attributed to the increased blood platelet numbers that may occur with vitamin E deficiency.[25]

Other in vitro tests have demonstrated that addition of vitamin E to human endothelial cell culture has no effect on PGI_2 production, whereas vitamin E addition to cell culture inhibits platelet TXA_2 synthesis.[10] Similarly, we have also reported that while synthesis of PGI_2 in lung was not affected, production of TXB_2 was decreased by vitamin E supplementation.[26] Salonen et al.[11] recently indicated a stronger association between a decrease in TXA_2 production and vitamin E supplementation than with Se supplementation. Our results also indicate that TXA_2 synthesis is more sensitive to vitamin E deficiency than Se deficiency.

Ex vivo synthesis of PGI_2 by aortic rings was affected by both dietary vitamin E and Se treatments. Ex vivo production of PGI_2 synthesis was not different between the two groups with deficiency of either nutrient (groups 2 and 3). In addition, synthesis of PGI_2 was not further decreased when the rats were deficient in both nutrients. However, in rats supplemented with vitamin E and Se (group 1), the ex vivo PGI_2 formation was significantly higher than other groups. Therefore, it appears that deficiency with one of these two nutrients may counterbalance the effect of supplementation with the other. Because PGI_2 synthetase activity can be irreversibly inhibited by alkyl hydroperoxides,[27,28] Schiavon et al.[9] suggested that the decreased level of hydroperoxides resulting from increased activity of GSH-Px with Se supplementation can lead to increased PGI_2 production. This situation might occur when the level of vitamin E in diet or in tissue is more than adequate (as it was in group 1), in which case changes in Se status would alter PGI_2 production. Selenium, via GSH-Px, controls the concentration of H_2O_2 and hydroperoxides within the cell and may thereby modulate the synthesis of PGI_2.[29] Increased bleeding time resulting from Se supplementation has been reported due to increased GSH-Px activity, which favors formation of PGI_2 over TXA_2.[30] This differential effect of Se on PGI_2 and TXA_2 production appears related to the higher sensitivity of PGI_2 synthetase to peroxides relative to TXA_2 synthetase.[31] Our data demonstrate that ex vivo synthesis of TXA_2 by clotting whole blood is more sensitive to vitamin E manipulation than to dietary changes in Se. Supplementation of diet with 0.2 ppm Se had no effect on TXB_2 production by clotting whole blood from either vitamin

E-deficient or -supplemented rats (group 1 versus group 2 or group 3 versus group 4).

The balance between the production of PGI_2 by arterial endothelial and fibroblast cells and TXA_2 by platelets is an important modulating factor in platelet activity and aggregation.[6] In this respect, our results show that rats supplemented with vitamin E and Se had a higher mean PGI_2:TXA_2 ratio than of those fed diets deficient in one or both of these nutrients (group 1 = 36.1, group 2 = 16.7, group 3 = 10.7, and group 4 = 11.7). These data demonstrate that dietary vitamin E and Se supplementation can increase the PGI_2:TXA_2 ratio, which in turn could decrease platelet aggregability. Furthermore, our data indicate that aortic synthesis of PGI_2 is sensitive to alterations in vitamin E and Se status, while platelet synthesis of TXA_2 is sensitive to changes in vitamin E levels only. Thus, vitamin E and Se play a specific but different role in controlling TXA_2 and PGI_2 synthesis. The relative increase in the PGI_2:TXA_2 ratio achieved by supplementing the diet with vitamin E and Se might have a favorable effect in reducing blood clotting and the risk of stroke and myocardial infarction. It is interesting to note that recent epidemiological studies demonstrate a negative correlation between the status of antioxidant nutrients and the risk of coronary heart disease.[32]

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