Vitamin E Suppresses Enhancement of Factor VIII-Dependent Thrombin Generation by Systemic Hypoxia

Jong-Shyan Wang, PhD; Mei-Ling Cheng, PhD; Hsiu-Chuan Yen, PhD; Bih-Show Lou, PhD; Huang-Chun Liu, MS

Background and Purpose—Increased thrombin activity is an essential component of hemostatic reactions. This study elucidates how various hypoxic interventions impact endogenous thrombin generation (TG) after treatment with/without lipophilic antioxidant vitamin E.

Methods—Twenty-four healthy sedentary men were randomly assigned to vitamin E (n = 12) and placebo (n = 12) groups. These subjects were randomly exposed to 12% (severe hypoxia), 15% (moderate hypoxia), 18% (light hypoxia), and 21% (normoxia) O2 for 2 hours in a normobaric hypoxia chamber. A novel calibrated, automated thrombinography approach was used to measure TG in plasma.

Results—in the placebo group, severe hypoxia enhanced plasma FVIII level/activity and TG, which was accompanied by increased urinary 15-F2t-8-isoprostane level and decreased plasma total antioxidant content and superoxide dismutase activity. However, depletion of FVIII by incubation with anti-FVIII antibodies in plasma suppressed enhancement of TG by severe hypoxia. After administration of 1000 IU vitamin E, severe hypoxia did not significantly alter urinary 15-F2t-8-isoprostane level and plasma total antioxidant content, superoxide dismutase activity, FVIII level/activity, or TG. Moreover, redox status, FVIII level/activity, and TG were constant in response to moderate hypoxia, light hypoxia, and normoxia in the placebo and vitamin E groups.

Conclusion—we conclude that severe hypoxia promotes FVIII-dependent TG, likely by elevating oxidative stress; this hypoxic effect was ameliorated by pretreatment with vitamin E. (Stroke. 2009;40:656-659.)

Key Words: coagulation • oxygen • tocopherol

Acute exposure to hypoxic environments typically shifts the endothelial phenotype toward that in which anticoagulant characteristics are reduced and proinflammatory features dominate the endovascular milieu,1 increasing the risk of venous thromboembolism. Blood undergoes oxidative stress during hypoxia,2 and elevated oxidative stress promotes the assembly of the prothrombinase complex and subsequently generates thrombin.3 Hence, we hypothesize that hypoxia affects endogenous thrombin generation (TG) through modulating the redox status in circulation, in which reactions are determined by the exposing concentrations of O2 in the air. To test this hypothesis, this study clarifies how various hypoxic interventions influence TG as well as lipid peroxidation and antioxidative capacity. Furthermore, the effect of vitamin E (a lipophilic antioxidant) on TG mediated by hypoxia was also investigated, thereby elucidating the role of oxidative stress on blood coagulation under various hypoxic conditions.

Methods

The Ethics Committee of Chang Gung Memorial Hospital approved this study. Informed consent was obtained from all subjects. Twenty-four healthy nonsmokers, who did not use medications or vitamins, were infection-free and cardiopulmonary risk-free, and did not have acute mountain sickness symptoms during experimental periods. Compliance rates were 100%.

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Results

The placebo and vitamin E groups did not differ significantly in their anthropometric data: age, 22.2±0.8 versus 23.2±0.9 years; height, 172.5±2.0 versus 171.6±1.9 cm; weight, 65.9±1.9 versus 66.5±1.5 kg, and body mass index, 22.3±0.7 kg/m² versus 22.8±0.8 kg/m², respectively. In the placebo group, SH decreased plasma total antioxidant content and superoxide dismutase activity, which was accompanied by a significant increase in urinary 15-F₂t-8-isoprostane level; total antioxidant content, superoxide dismutase activity, and 15-F₂t-8-isoprostane level were unchanged in response to MH, LH, and N. Conversely, in the vitamin E group, no significant change in the redox status existed after all hypoxic interventions (Table 1).

Although SH in the placebo group did not influence lag time of thrombin formation and time to thrombin peak, the ETP and thrombin peak height increased after this hypoxic intervention. However, SH in the vitamin E group was not associated with any changes in all TG-related parameters.

Moreover, no significant alteration in dynamic TG existed after MH, LH, and N exposures in the 2 groups (Table 2).

The SH in the placebo group increased FVIII level/activity in plasma; no significant change occurred in plasma FVIII level/activity after all hypoxic exposures in the vitamin E group (Table 3). Treating plasma with anti-FVIII antibodies reduced ETP and thrombin peak height and extended time to thrombin peak under all experimental conditions, simultaneously limiting increases in ETP and thrombin peak height by SH in the placebo group (Table 2).

Discussion

Extremely hypoxic environments are associated with increased incidence of vascular thromboembolic events. The authors’ recent work established that acute exposure to 12% O₂, but not 15% O₂, suppressed vascular endothelial function and impaired hemodynamic control, likely by reducing antioxidative capacity and nitric oxide bioavailability. Clinical investigations have also demonstrated that elevated oxidative stress facilitates oxidation of plasma lipoprotein, promoting the assembly of the prothrombinase complex and thrombin production, further causing blood to approach a procoagulation state. Therefore, SH may simultaneously increase vessel resistance, reduce blood flow, and enhance blood coagulability by promoting lipid peroxidation and hindering antioxidative capacity in circulation, which, in turn, increase risk of vascular thrombosis.

Vitamin E, a lipophilic antioxidant, prevents tissue injury caused by free oxygen radicals through the effect of chain breaking on lipid peroxidation. Dietary supplementation with vitamin E has been demonstrated to increase the tendency to bleed by inhibiting intrinsic coagulation as well as by protecting against the detrimental effects of lipoprotein oxidation by limiting thrombin formation. In this work, pretreatment with vitamin E suppressed the triggering of lipid peroxidation by SH and reduced the increases to plasma FVIII level/activity and TG after this hypoxic stimulation. When FVIII in plasma was depleted, SH did not change TG after treatment with or without vitamin E. These experimental
observations clearly verify that increases in plasma FVIII bioactivity by SH-enhanced oxidative stress, which activates intrinsic coagulation, further promotes thrombin production.

A clinical investigation has demonstrated that patients with sleep apnea have lower vitamin E level and antioxidative capacity than healthy control subjects. Moreover, changes of plasma redox status by episodic hypoxia in these patients were associated with cardiovascular dysfunction. However, the antithrombotic benefits for the clinical uses of vitamin E supplement need to be further investigated by large Phase III trials.

| Table 2. Comparison of Dynamic Thrombin Generation in Intact and FVIII-Depleted Plasma Between Placebo and Vitamin E Groups During Experimental Periods |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | N               | LH              | MH              | SH              |                 |                 |
|                  | Pre             | Post            | Pre             | Post            | Pre             | Post            |
| Lag time, minutes |                 |                 |                 |                 |                 |                 |
| Placebo          | 2.2 (2.1–2.3)   | 2.1 (2.0–2.2)   | 2.2 (2.1–2.4)   | 2.1 (2.1–2.4)   | 2.1 (2.0–2.2)   | 2.2 (2.1–2.3)   |
| Vitamin E        | 2.1 (2.0–2.2)   | 2.2 (2.1–2.3)   | 2.2 (2.1–2.3)   | 2.2 (2.2–2.4)   | 2.2 (2.1–2.3)   | 2.2 (2.1–2.3)   |
| ETP, nM/min      |                 |                 |                 |                 |                 |                 |
| Placebo          | 1480 (1382–1552)| 1468 (1372–1532)| 1441 (1380–1542)| 1482 (1389–1582)| 1480 (1385–1580)| 1455 (1355–1535)|
| Vitamin E        | 1505 (1405–1590)| 1475 (1380–1501)| 1485 (1402–1521)| 1505 (1405–1622)| 1486 (1386–1557)| 1465 (1382–1520)|
| Peak height, nM  |                 |                 |                 |                 |                 |                 |
| Time to peak, minutes |                 |                 |                 |                 |                 |                 |
| Placebo          | 5.1 (4.8–5.2)   | 4.9 (4.7–5.1)   | 5.0 (4.8–5.2)   | 5.0 (4.7–5.2)   | 5.0 (4.8–5.1)   | 4.9 (4.8–5.2)   |
| Vitamin E        | 5.0 (4.7–5.2)   | 5.0 (4.8–5.2)   | 5.0 (4.7–5.2)   | 5.0 (4.8–5.2)   | 5.0 (4.8–5.1)   | 4.9 (4.8–5.2)   |

Peak height indicates time until initial thrombin formation. Values are median (interquartile range).

*P<0.05, pre versus post.
†P<0.05, placebo versus vitamin E.

Peak height indicates height of thrombin peak; Time to peak, time to thrombin peak; Pre, preintervention; Post, postintervention.

| Table 3. Comparison of FVIII Level and FVIII:C Activity Between Placebo and Vitamin E Groups During Experimental Periods |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | N               | LH              | MH              | SH              |
| FVIII level, ng/mL |                 |                 |                 |                 |
| Placebo          | 173 (132–191)   | 176 (141–200)   | 175 (130–193)   | 178 (142–203)   |
| Vitamin E        | 158 (114–176)   | 165 (133–185)   | 156 (118–178)   | 161 (135–181)   |
| FVIII:C activity, % |                 |                 |                 |                 |
| Placebo          | 96 (82–108)     | 96 (86–112)     | 95 (83–106)     | 95 (83–106)     |
| Vitamin E        | 93 (81–109)     | 97 (80–108)     | 94 (82–109)     | 94 (82–104)     |

Values are median (interquartile range).

*P<0.05, pre versus post.
†P<0.05, placebo versus vitamin E.

Pre indicates preintervention; Post, postintervention.
cardiopulmonary trials. Results of this study provide possible valuable insight into the protective effects of antioxidant vitamin E, which reduces the risk of thrombosis associated with hypoxic exposure.

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Disclosures

None.

References

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