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Rapid Letter

Selenium Modulates 1-*O*-Alkyl-2-Acetyl-*sn*-Glycero-3-Phosphocholine (PAF) Biosynthesis in Bovine Aortic Endothelial Cells

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ABSTRACT

Selenium (Se) deficiency has been reported to increase platelet-activating factor (PAF) production in human endothelial cells; however, the mechanism is unclear. This study demonstrated that tumor necrosis factor-α (TNF-α) stimulated Se-deficient bovine aortic endothelial cells (BAEC) produced significantly more PAF than Se-supplemented cells. Moreover, the increase in the level of PAF was associated with enhanced activity of two anabolic enzymes in the remodeling pathway: phospholipase A_2 and Lyso-PAF:acetyl-coenzyme A acetyltransferase (Lyso-PAF-AcT). In contrast, the activity of the PAF catabolic enzyme, PAF-acetylhydrolase, was not affected by Se status. Interestingly, prostacyclin, a potent vasodilator and inhibitor of platelet aggregation, inhibited the activity of Lyso-PAF-AcT and reduced the PAF production in TNF-α-stimulated BAEC. Therefore, we conclude that Se deficiency alters PAF production in TNF-α-stimulated BAEC by altering the activity of anabolic enzymes involved in the remodeling pathway partially through the inhibition of prostacyclin production. Antioxid. Redox Signal. 3, 1147–1152.

INTRODUCTION

PLATELET-ACTIVATING FACTOR (PAF) is a potent activator of endothelial cells (EC), platelets, and many inflammatory cells. Interest has grown concerning the potential role of PAF in the development of vascular disorders. Selenium (Se) nutrition may have a profound impact on vascular health (2, 16, 19), and Se deficiency has been reported to increase the formation of PAF in human EC (7, 8). Consequently, some of the deleterious effects of Se deficiency on EC function may be the result of altered PAF production. The ability of Se to inhibit the formation of PAF may be due in part to Se's role as an integral component of the intracellular enzyme Se-dependent glutathione peroxidase (Se-GSH-Px), which protects cells against peroxidation and modulates intracellular peroxide levels (16). The mechanisms involved in increased PAF formation in Se-deficient EC are unclear; however, they may involve alterations in the PAF synthetic pathways. PAF is derived from the phospholipids of cell membranes and is synthesized via two independent enzymatic pathways: the *de novo* pathway and the remodeling pathway. The *de novo* pathway forms endogenous PAF required for physiological functions in resting cells. In the remodeling pathway, phospholipase A_2 (PLA_2) catalyzes the structural modification of a membrane lipid (1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine) to produce

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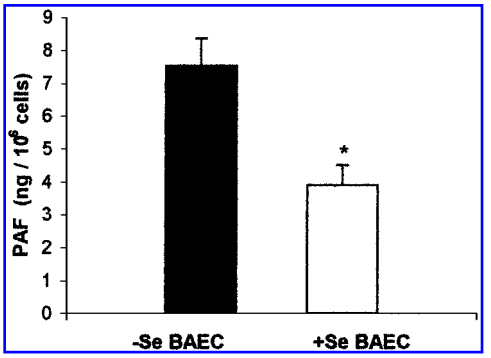

FIG. 1. Effect of Se status on PAF levels in TNF-α-stimulated BAEC. Cells were cultured in −Se or +Se media and stimulated with TNF-α (10 ng/ml) for 12 h. PAF was purified by HPLC and quantified as described in Materials and Methods. Data are expressed as means ± SD of three independent experiments and were analyzed using the Student's *t*-test. *Significantly different ($p < 0.05$) compared with −Se group.

lyso-PAF. The enzyme Lyso-PAF:acetyl-coenzyme A acetyltransferase (Lyso-PAF-AcT) then introduces an acetyl residue from acetyl-coenzyme A at the *sn*-2 position of the glycerol backbone, yielding the vasoactive PAF. Conversely, PAF-acetylhydrolase (PAF-AH), which cleaves the acetyl chain at the *sn*-2 position, plays a major role in inhibiting PAF activity. The remodeling pathway is responsible for PAF biosynthesis in response to cellular stimulation by agonists, including histamine, oxidants, and inflammatory cytokines such as tumor necrosis factor-α (TNF-α) (11, 14).

TNF-α is known to induce the formation of reactive oxidant species (ROS). Furthermore, it is known that ROS can alter PAF and eicosanoid formation such as prostacyclin (PGI_2) in many cells, including EC (3, 5, 17). Because Se can function as an antioxidant, it plays a vital role in the management of oxidative stress during inflammation. We hypothesize that oxidant stress, a consequence of Se status, will affect the production of PAF in TNF-α-stimulated bovine aortic EC (BAEC) by modifying the activity of key enzymes involved in PAF synthesis.

MATERIALS AND METHODS

Reagents

1-Stearoyl-2-[1-^{14}C]arachidonyl-*sn*-glycero-3-phosphocholine (specific radioactivity, 55.0 mCi/mmol), [1-^{14}C]acetyl-coenzyme A (52.0 mCi/mmol), and 5-hydroxy[G-^{3}H]tryptamine creatine sulfate (19.9 mCi/mmol) were purchased from Amersham Life Science Products (Arlington Heights, IL, U.S.A.). 1-*O*-Hexadyl-2-acetyl-*sn*-glycero-3-phosphocholine (PAF) was obtained from Calbiochem (La Jolla, CA, U.S.A.). The PAF-AH assay kit, carbaprostacyclin, and PGI_2 were purchased from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.). Low-Se (<10 ng/ml) fetal bovine serum was purchased from Hyclone Laboratory (Clifton, NJ, U.S.A.). Dichlorodihydrofluorescein diacetate (DCF-DA) was purchased from Molecular Probes (Eugene, OR, U.S.A.). Precoated silica gel G 150A plates were purchased from Whatman Laboratory (Clifton, NJ, U.S.A.). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Preparation of Se-deficient (−Se) and Se-adequate (+Se) BAEC culture

We utilized a unique culture system for the development of −Se primary BAEC, which was based on a low-serum culture protocol previously published (1, 15). Primary −Se BAEC were subcultured in F12K medium containing 3% fetal bovine serum, antibiotics and antimycotics (100 U/ml), L-glutamine (2 m*M*), heparin (100 μg/ml), insulin (10 μg/ml), transferrin (5 μg/ml), and linoleic acid (1 μg/ml), and

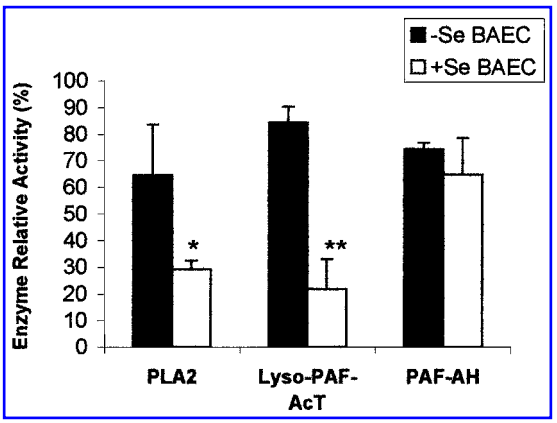

FIG. 2. Effects of altered Se status on the activity of enzymes in the PAF remodeling pathway. The activities of PLA_2, Lyso-PAF-AcT, and PAF-AH were assayed as described under Materials and Methods. Values are means ± SD from three independent experiments, and analysis was done using the Student's *t* test. *Significantly different ($p < 0.05$) compared with −Se group; **significantly different ($p < 0.01$) compared with −Se group.

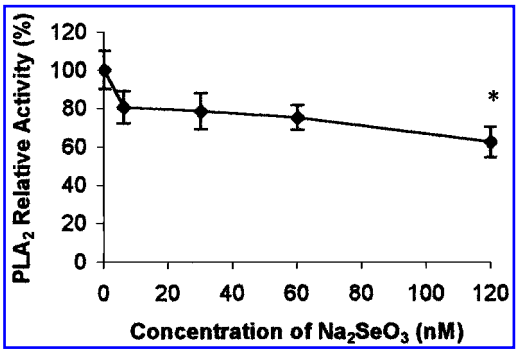

FIG. 3. ***In vitro*** **inhibition of PLA_2 activity by Se (Na_2SeO_3).** The homogenates from −Se and +Se BAEC were preincubated with different concentrations of Na_2SeO_3 for 60 min at room temperature. The activity of PLA_2 was calculated from the amount of radioactivity associated with the arachidonic acid fraction after the reaction (30 min). Values depicted are means ± SD from three individual experiments, and analysis was done using the Student's *t* test. *Significantly different ($p < 0.05$) from control group (did not add Na_2SeO_3).

+Se BAEC were concurrently cultured in the same medium in the presence of 60 n*M* sodium selenite.

Measurement of Se-GSH-Px activity

The Se-GSH-Px activity of BAEC was determined by a coupled-enzymatic assay in which the oxidation of NADPH was monitored spectrophotometrically and peroxidase activity was determined using H_2O_2 as a substrate (15). One unit of activity was defined as the amount of enzyme oxidizing 1 μmol of NADPH per minute.

Measurement of intracellular ROS production

Intracellular ROS production was determined by incubating BAEC for 15 min at 37°C with the peroxide-sensitive fluorophore DCF-DA. Fluorescence was determined by flow cytometry using the Epics XL (Coulter) system; with excitation at 513 nm and emission at 530 nm. Values are expressed as arbitrary units of fluorescence.

PAF extraction and purification

PAF in EC is routinely extracted by a modification of the procedure of Pinckard (12) and purified by HPLC as described by Scappaticci *et al*. (13). In brief, BAEC were cultured in −Se and +Se media until confluency. Cells were stimulated with TNF-α (10 ng/ml) for 12 h. After stimulation, −Se and +Se cells (1×10^7) were resuspended in 2 ml of chloroform/1 ml of acetic acid-acidified methanol (50 m*M*)/1.5 ml of (0.1 *M*) sodium acetate solution and vortexed. After phase separation, the lower phase (chloroform phase) was dried under a nitrogen stream and the residual was dissolved in 0.2 ml of HPLC solution A (acetonitrile/methanol/acetic acid/water, 520: 20: 0.5: 7). The samples were injected into an HPLC system for purification.

PAF quantification

PAF was quantified by the degranulation assay as described by Muller and Nigam (9). In brief, bovine blood (100 ml) was collected and centrifuged to form platelet-rich plasma. [^{3}H] Tryptamine (serotonin) was added to the platelet-rich plasma (1 μCi [^{3}H]tryptamine/1 ml of platelet-rich plasma) and incubated for 30 min at 37°C. Following labeling, the platelets were resuspended in Tyrode's buffer, pH 7.2. [^{3}H]Tryptamine release was measured by mixing 250 μl of prewarmed labeled platelet suspension ($0.5–1 \times 10^8$ platelets/ml) with 10 μl of PAF standard or sample that had been solubilized in a saline

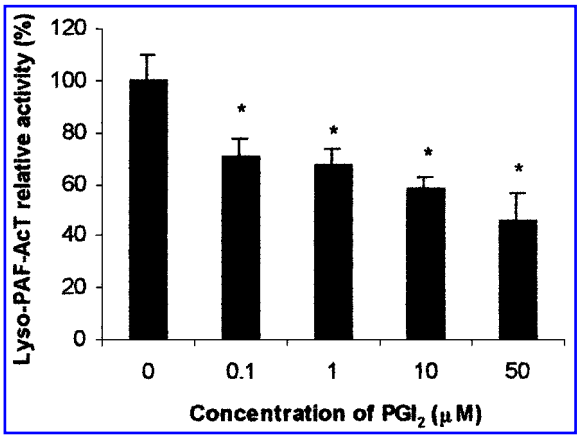

FIG. 4. ***In vitro*** **inhibition of Lyso-PAF-AcT activity by PGI_2.** The homogenates from −Se BAEC were preincubated with different concentrations of PGI_2 (0. 1–50 μM) for 30 min at room temperature. The activity was calculated from the amount of radioactivity associated with the PAF fraction after TLC isolation. Values depicted are means ± SD of three independent experiments, and analysis was done using the Student's *t* test. *Significantly different ($p < 0.05$) compared with control group (did not add PGI_2).

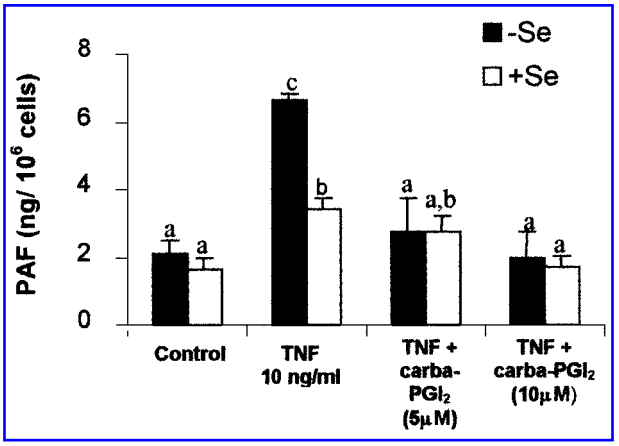

FIG. 5. The effect of carbaprostacyclin on TNF-α stimulated PAF synthesis in BAEC. Se-deficient and Se-supplemented BAEC in culture were preincubated with various doses of carbaprostacyclin (5 and 10 μM) or solvent vehicle (control) for 1 h, then TNF-α (10 ng/ml) was added, and the cell cultures were incubated for 12 h before extraction. PAF in the cell extract was purified by HPLC and quantified as described previously. Data are expressed as means ± SD of three independent experiments and were analyzed using the Student's t test. Bars with different letters are significantly different ($p < 0.05$) within carbaprostacyclin treatment groups and between carbaprostacyclin treatment groups.

solution (2.5 mg of bovine serum albumin/ml) in a polypropylene vial. The vials were incubated at 37°C for 2 min. PAF levels were measured by liquid scintillation spectrometry (Backman LS 60001C counter).

PLA_2 assay

PLA_2 activity in −Se or +Se BAEC homogenates was determined by measuring the radioactivity of arachidonic acid released from 1-stearoyl-2-[1-^{14}C]arachidonyl-*sn*-glycero-3-phosphocholine as previously described (4).

PAF-AH assay

TNF-α (10 ng/ml, 12 h)-stimulated BAEC were harvested from both −Se and +Se culture media. Cells (4×10^6) were resuspended in 2 ml of fresh culture media and homogenized by sonication (Branson sonifier 250). The cell homogenates were then centrifuged at 10,000 g for 15 min at 4°C. The PAF-AH activity was measured using the PAF-AH Assay Kit (Cayman Chemical Co.) according to the manufacturer's instructions.

Lyso-PAF-AcT assay

Lyso-PAF-AcT activity was examined by the modified method of Nakagawa *et al*. (10). In brief, the assay mixture (1 ml, 0.05 mM Tris-HCl buffer, pH 7.0) contained 200 nmol of [1-^{14}C]acetyl coenzyme A (specific radioactivity, 5,500 dpm/nmol), 100 nmol of Lyso-PAF, and BAEC homogenate (2.5×10^6 cells, which was stimulated with 10 ng/ml TNF-α for 12 h before homogenization).

RESULTS AND DISCUSSION

The present study demonstrated that −Se BAEC produced significantly more PAF than +Se cells when stimulated with TNF-α (Fig. 1). Similar increases in PAF production have been found in −Se human umbilical vein EC; however, the mechanism responsible for this change remains unclear (7). Our findings revealed that the activities of key anabolic enzymes in the remodeling pathway, PLA_2 and Lyso-PAF-AcT, were significantly enhanced in −Se BAEC stimulated with TNF-α (Fig. 2). The present study also demonstrated that the activity of the PAF catabolic enzyme, PAF-AH, was not affected by Se status (Fig. 2). Together these results suggest that Se deficiency enhances PAF biosynthesis by increasing the activity of anabolic enzymes required for PAF synthesis in the remodeling pathway without affecting the degradation of newly formed PAF. These findings are important because previous research has shown that the concurrent activation of both anabolic enzymes is required for PAF synthesis (6).

Using increasing levels of Se, we determined that the addition of Na_2SeO_3 directly into −Se BAEC homogenates at concentrations ranging from 6 to 60 nM did not affect PLA_2 activity (Fig. 3). Se-supplemented cells in our culture system receive 60 nM Na_2SeO_3; therefore, it is unlikely that Se levels in our culture system are sufficient to inhibit PLA_2 activity directly. However, Se can be incorporated into a number of selenoproteins whose biological activity may play a role in altering the activity of en-

zymes involved in PAF synthesis. In this study, Se-GSH-Px activity in −Se BAEC is sixfold less than that observed in +Se BAEC [4.58 + 2.96(−Se) vs. 30.6 + 3.34 (+Se) mU/(3×10^6 cells)]. Furthermore, −Se BAEC were determined to be oxidatively stressed as indicated by the increased formation of intracellular ROS [13.9 + 3.4 (−Se) vs. 6.7 + 1.4 (+Se), arbitrary units of fluorescence]. Increased oxidative stress as a result of reduced Se-GSH-Px activity can influence the formation of other lipid membrane-derived metabolites; for example, PGI_2 production is significantly decreased in activated −Se BAEC (17). Furthermore, PGI_2 has been reported to inhibit PAF formation in human vascular EC (20). Thus, the increased PAF production seen in BAEC partially could be due to diminished PGI_2 levels in TNF-α-stimulated −Se BAEC.

To verify this premise, we examined the activity of Lyso-PAF-AcT in response to the addition of increasing amounts of exogenous PGI_2 into −Se BAEC homogenates. Interestingly, PGI_2 inhibited the activity of Lyso-PAF-AcT in a dose-dependent manner in −Se BAEC (Fig. 4). Furthermore, carbaprostacyclin, a PGI_2 analogue, decreased TNF-α-stimulated PAF production in intact BAEC in a dose-dependent manner (Fig. 5). Therefore, it is conceivable that diminished PGI_2 production in −Se BAEC is partially responsible for the alterations in PAF production observed in this study because in the absence of PGI_2, Lyso-PAF-AcT activity is higher in activated −Se BAEC.

This is the first report to determine the effects of Se status on Lyso-PAF-AcT activity in EC. Accordingly, this enzymatic regulation could constitute an important control mechanism for the maintenance of PAF levels in BAEC. It should be noted that in addition to the formation of Lyso-PAF, lysophosphatidylcholine and other lysophospholipids also could be produced upon PLA_2 activation. High concentrations of lysophospholipids have been shown to be cytolytic because of their detergent nature (18). Therefore, the potential role of Se as a nutrient regulator in the formation of lysophospholipids merits further study. Our research provides new insight into the specific cellular mechanism involved in the regulation of PAF production by Se status.

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ABBREVIATIONS

BAEC, bovine aortic endothelial cells; DCF-DA, dichlorodihydrofluorescein diacetate; EC, endothelial cells; Lyso-PAF-AcT, Lyso-PAF:acetyl-coenzyme A acetyltransferase; PAF, platelet-activating factor; PAF-AH, PAF-acetylhydrolase; PGI_2, prostacyclin; PLA_2, phospholipase A_2; ROS, reactive oxygen species; Se, selenium; Se-GSH-Px, selenium-dependent glutathione peroxidase; TNF-α, tumor necrosis factor-α.

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