PROSTAGLANDINS LEUKOTRIENES AND ESSENTIAL FATTY ACIDS

Prostaglandins Leukotrienes and Essential Fatty Acids (1991) 44, 89–92

Inhibition of PGE_2 Production in Macrophages from Vitamin E-treated Rats

W. Sakamoto, K. Fujie, J. Nishihira*, M. Mino†, I. Morita‡ and S.-I. Murota‡

*Department of Biochemistry, School of Dentistry and *School of Medicine, Hokkaido University, Sapporo, Japan, †Department of Pediatrics, Osaka Medical College, Daigaku-cho, Takatsuki, Osaka, Japan, and ‡Section of Physiological Chemistry, Institute of Stomatognathic Sciences, Tokyo Medical and Dental University, Tokyo, Japan (Reprint requests to WS)*

ABSTRACT. **Rat peritoneal macrophages from vitamin E-treated rats (5 mg per rat for 6 successive days) contained 403.3 ± 90.7 ng α-tocopherol/10^6 cells, whereas control macrophages contained 1.2 ± 0.4 ng. PGE_2 production in the macrophages from vitamin E-treated rats was significantly suppressed when stimulated with PMA and calcium ionophore A23187. The mechanism of vitamin E inhibition of PGE_2 production in macrophages was investigated. The release of (^{14}C)-arachidonic acid from pre-labeled macrophages and the conversion of (^{14}C)-arachidonic acid to PGE_2 by the homogenate of the cells were remarkably reduced. These results strongly suggested that the inhibition of PGE_2 production by vitamin E results from the inhibition of the activities of both phospholipase A_2 and cyclooxygenase.**

INTRODUCTION

Macrophages play a key role in inflammatory reactions and release a variety of inflammatory mediators including prostaglandins, leukotrienes, and hydroxy fatty acids (1, 2). These mediators are synthesized from arachidonic acid which is stored in the membrane phospholipids of macrophages (3). Recently, vitamin E, which functions as a physiological antioxidant for membrane lipid, has been shown to reduce thromboxane A_2 synthesis in thrombin-stimulated rat platelets (4) and 5-lipoxygenase products in calcium ionophore A23187-stimulated rat polymorphonuclear leucocytes (5). However, the inhibitory action on such eicosanoids synthesis in the macrophages by vitamin E is not clear. Previously, we reported that the content of vitamin E was extremely high in peritoneal macrophages when vitamin E was inoculated by intraperitoneal injection for 6 successive days in rats (6). The present study was undertaken to examine the mechanism of the in vivo inhibition of PGE_2 production in macrophages isolated from vitamin E-treated rats.

Date received 13 November 1990
Date accepted 25 April 1991

MATERIALS AND METHODS

Animals

Male Wistar rats weighing approximately 350 g were used throughout the work. Five mg vitamin E or placebo vehicle (0.1 ml) were injected intraperitoneally for 6 successive days, and rats were killed on the day after the final injection. Vitamin E (DL-α-tocopherol) and placebo vehicle (polyethylene 60-hydrogenerated castor oil) were gifts from Eisai Co, Japan.

Chemicals

The following commercial materials were used: PMA and A23187 from Sigma Chemical Co (USA); synthetic 5-hydroxyeicosatetraenoic acid, prostaglandin $F_{2\alpha}$ and prostaglandin E_2 from Ono Pharmaceutical Co (Japan); thin-layer plate (silica 60) from Merck (Darmstadt, FRG); (^{14}C)-arachidonic acid (specific activity 60 mCi/mmol) from Amersham (UK); RPMI 1640 and Hanks balanced salt solution (HBSS) from Nissui Pharmaceutical Co (Japan); bovine fetal calf serum from Boehringer Mannheim GmbH (FRG). All other chemicals were of the highest grade available commercially.

Measurement of α-tocopherol

Measurement of α-tocopherol in macrophages was determined, as described previously (7).

Isolation of peritoneal macrophages

Peritoneal exudate cells were harvested by washing the peritoneal cavity with 10 ml of acid-citrate-dextrose solution after killing the rats and cultured in RPMI 1640 containing 10% fetal calf serum, as described previously (7). After incubation at 37°C for 2 h in a 5% CO_2-air incubator, the adherent cells were used as peritoneal macrophages.

Measurement of PGE_2 production

Macrophages (1.2–2.8 × 10^6 cells) were cultured in 2 ml of RPMI 1640 containing 10% fetal calf serum on plastic dishes (Falcon®, 10 × 35 mm) under conditions described above. After incubation for 2 h, each dish was washed with 2 ml of RPMI 1640, and then washed with 2 ml of HBSS. The macrophages were preincubated with 2 ml of HBSS for 5 min at 37°C. The reaction was carried out for 30 min by an addition of 139 nM PMA and/or 1 μM A23187. After incubation, 100 μl of the medium was transferred into 1.0 ml of HBSS containing indomethacin (10 μg/ml), and centrifuged at 10 000 rpm for 5 min. The amount of PGE_2 in the supernatant was determined by radioimmunoassay with an PGE_2 (^{125}I)-RIA Kit (Du pont de Nemours Inc, Boston, USA).

(^{14}C)-arachidonic acid release of labeled macrophages

To measure arachidonic acid release, macrophages were cultured in dishes (Falcon®, 10 × 35 mm) in 2 ml of RPMI 1640 containing 10% fetal calf serum, penicillin (1 U/ml), streptomycin (5 mg/ml), and (^{14}C)-arachidonic acid (0.1 μCi/2 ml per dish) for 18 h under 5% CO_2-air incubator at 37°C. The labeled cells were washed twiced with 2 ml of HBSS, and suspended in 2 ml of HBSS. After incubation with PMA (139 nM) or A23187 (1 μM) for 30 min under 5% CO_2-air incubator at 37°C, the medium was collected and centrifuged at 2000 rpm for 10 min to remove all cellular materials. Radioactivity of the 0.5 ml supernatant was counted by liquid scintillation counter.

Prostaglandin synthesis by the homogenate of macrophages

Macrophages (1.4–5.6 × 10^6 cells) were disrupted by sonication and resulting homogenates were used for the assay of prostaglandin synthesis, as described previously (8). Briefly, 0.5 ml of the homogenates was incubated with 0.2 μCi of (^{14}C)-arachidonic acid for 15 min at 37°C. Prostaglandins were extracted with ethyl acetate at pH 3.0 and analyzed by thin-layer chromatography. The solvent system was chloroform/methanol/acetic acid/water (90:8:1:0.8, v/v). The radioactivities were measured by a radiochromatoscanner.

RESULTS

PGE_2 production of the macrophages isolated from vitamin E-treated rats

The α-tocopherol content of the macrophages from vitamin E-treated rats was 403.3 ± 90.7 ng/10^6 cells, while the control macrophages contained 1.2 ± 0.4 ng/10^6 cells. As described in Table 1, PGE_2 production by the control macrophages as stimulated with 139 nM PMA and 1 μM A23187 was 1.11 ± 0.12 and 11.52 ± 2.48 ng/10^6 cells/30 min, respectively, while unstimulated macrophages produced 0.39 ± 0.10 ng/10^6 cells/30 min. On the other hand, the macrophages from vitamin E-treated rats show less PGE_2 production than those from the controls. The complete inhibition of the action of PMA was observed, resulting in synthesis being down to the level of the unstimulated macrophages. When stimulated with A23187, the production of PGE_2 was reduced to approximately 10% of the control.

Table 1 In vivo inhibition of PGE_2 synthesis by macrophages from vitamin E-treated rats

	PGE_2 synthesis (ng/10^6 cells/30 min)		
	Stimulus		
	None	PMA	A23187
Placebo (n=3)	0.39 ± 0.10	1.11 ± 0.12	11.52 ± 2.48
Vitamin E (n=3)	0.32 ± 0.08	0.31 ± 0.10	1.39 ± 0.17

Macrophages pooled 3–5 rats were used to assay PGE_2 production. Macrophage monolayers (1.2–2.8 × 10^6 cells) were stimulated with A23187 (1 μM) and PMA (139 nM) and then incubated for 30 min at 37°C. The amount of PGE_2 in the culture media was determined by radioimmunoassay. Other experimental conditions were described in the text. Values are expressed as the mean ± standard deviation (n = 3).

Arachidonic acid release in the macrophages isolated from vitamin E-treated rats

In order to clarify the inhibitory mechanism of PGE_2 production of the macrophages from vitamin E-treated rats, the release of (^{14}C)-arachidonic acid from pre-labeled macrophages was investigated. The release of (^{14}C)-arachidonic acid from the macrophages of vitamin E-treated rats scarcely occurred in response to stimulation with PMA and A23187, whereas the control macrophages released ap-

Table 2 (^{14}C)-arachidonic acid release from macrophages stimulated with A23187 and PMA

	(^{14}C)-arachidonic acid release (cpm/10^6 cells/30 min)		
	Stimulus		
	None	PMA	A23187
Placebo (n=3)	1736 ± 212	3671 ± 224	13668 ± 1052
Vitamin E (n=3)	1665 ± 224	1672 ± 202	2357 ± 152

Macrophage monolayer (0.7–1.1 × 10^6 cells) were preincubated with 0.1 μCi of (^{14}C)-arachidonic acid as described in the text. The cells labeled with (^{14}C)-arachidonic acid were stimulated with A23187 and PMA. After 30 min, the culture media were removed and analyzed for (^{14}C)-arachidonic acid release as described in the text. Values are expressed as the mean ± standard deviation (n=3).

proximately 2- to 8-fold (^{14}C)-arachidonic acid following stimulation as compared with the stimulated state (Table 2).

Prostaglandin synthesis by the homogenate of the macrophages isolated from vitamin E-treated rats

To measure the cyclooxygenase activity in the macrophages from vitamin E-treated rats, we examined

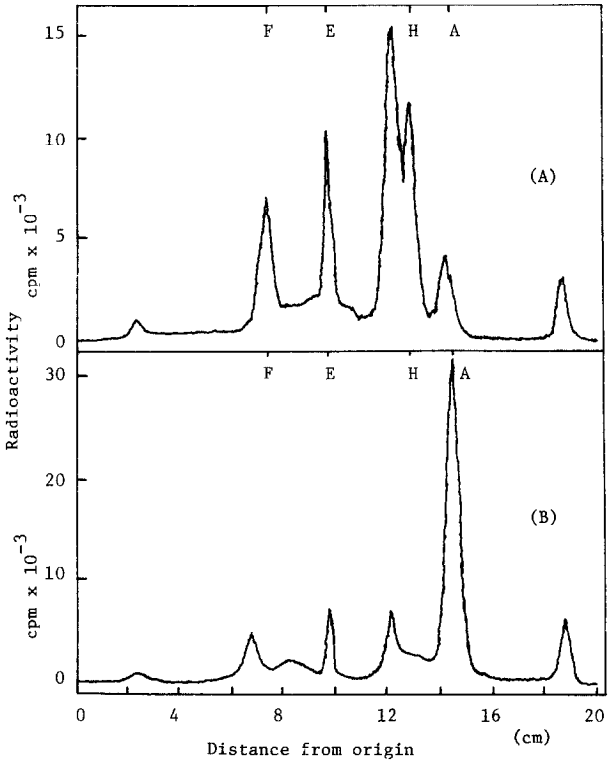

Figure Typical scanning profiles of prostaglandin synthesis by homogenate of macrophages from placebo and vitamin E-treated rats.

The cell homogenates (1.4 × 10^6 cells) were used for the assay of the prostaglandin synthesis as described in the text. (A) placebo; (B) vitamin E. Abbreviations: A, arachidonic acid; H, 5-hydroxyeicosatetraenoic acid; E, prostaglandin E_2; F, prostaglandin $F_{2\alpha}$. Similar results were obtained in two other individual experiments.

PGE_2 production by the homogenates of the cells. As shown in the Figure, conversion of (^{14}C)-arachidonic acid to PGE_2 by the homogenate of macrophages from vitamin E-treated rats was significantly reduced as compared with that of the controls. The production of PGE_2 from (^{14}C)-arachidonic acid in the control macrophages was 74 μg/10^6 cells/15 min, but was 40 μg/10^6 cells/15 min in the vitamin E-treated ones.

DISCUSSION

The present paper demonstrated that vitamin E is capable of attenuating the production of PGE_2 production in macrophages stimulated with PMA and calcium ionophore. It is widely accepted that PGE_2 synthesis is limited by the availability of free arachidonic acid which is liberated from membrane phospholipids by phospholipase A_2 (9). Activation of phospholipase A_2 is considered to be regulated by protein kinase C and the increase in the concentration of intracellular Ca^{2+} (10, 11). Recently, Pfannkuche et al demonstrated that mouse peritoneal macrophages stimulated by PMA produced prostaglandin through activation of protein kinase C(12). We also demonstrated that PMA stimulated PGE2 production from rat peritoneal macrophages, and that PGE_2 production in the vitamin E-treated rat macrophages was completely inhibited as stimulated with PMA. In spite of the stimulation with PMA, the release of (^{14}C)-arachidonic acid from labeled macrophages did not occur in the macrophages from vitamin E-treated rats, containing approximately 403 ng α-tocopherol/10^6 cells. These results suggested that the inhibition of PGE_2 synthesis by vitamin E might reflect the inhibition of phospholipase A_2 activity through protein kinase C activation. In fact, Mahoney and Azzi reported that vitamin E inhibited protein kinase C from bovine brain (13). We also demonstrated that macrophages isolated from vitamin E-treated rats had less protein kinase C activity (6). On the other hand, the production of PGE_2 and the release of (^{14}C)-arachidonic acid were also significantly reduced in the vitamin E-treated rat macrophages as stimulated with A23187. Most recently, several investigators reported activation of phospholipase A_2 by Ca^{2+} in the brain (14) and macrophages (15). Therefore, the inhibition of PGE_2 production in macrophages from vitamin E-treated rats seems to be due to the inhibition of phospholipase A_2. This result is in agreement with the reports on rat platelets and polymorphonuclear leucocytes by Chan et al (5, 16). Concerning the cyclooxygenase-PGE_2 synthetase, the conversion of arachidonic acid to PGE_2 by homogenates from vitamin E-treated rats was significantly reduced as compared with that of the con-

trols. This reduction may reflect the inhibition of cyclooxygenase by vitamin E, probably by removing an intermediate radical essential to the cyclooxygenase mechanism. Such a mechanism was demonstrated for phenol and α-naphthol of antioxidants by Hemler and Lands (17).

In conclusion, the present paper demonstrated that in vivo inhibition of PGE_2 production by vitamin E resulted from the inhibition of not only phospholipase A_2 but also cyclooxygenase in the macrophages. Since it has been suggested that oxidized low density lipoprotein could contribute to the progression of atheroma by stimulating arachidonate metabolism during incorporation into macrophages (18), treatment with high doses of vitamin E may have a beneficial effect in the earliest lesions of atherosclerosis.

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