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Polyunsaturated fatty acids reduce pyrogen-induced tissue factor expression in human monocytes

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Abstract—Endotoxin (LPS) and interleukin-1 β (IL-1β) increased the expression of tissue factor, a membrane-anchored glycoprotein that initiates blood coagulation on the surface of cultured human umbilical vein endothelial cells (HUVEC) and human monocyte/macrophages. On monocyte/macrophages, oleic acid strongly inhibited LPS-induced tissue factor expression, a similar activity also being obtained with regard to the pyrogenic effects of IL-1β. Other polyunsaturated fatty acids such as linoleic or linolenic acid also reduced tissue factor expression whereas palmitic acid was ineffective. In contrast, these compounds showed no effect on LPS- or IL-1β-induced tissue factor expression in HUVEC when tested at the concentration of 10 μM. These data therefore suggest that the well-recognized antithrombotic and antiatherogenic effect of polyunsaturated fatty acids may in part be mediated through an inhibition of tissue factor expression in monocyte/macrophages.

Key words: tissue factor; IL-1β; endotoxin; monocytes; macrophages; endothelium

TF* is an ubiquitous membrane-anchored glycoprotein that initiates blood coagulation by forming a complex with circulating factors VII and VIIa [1]. Under normal circumstances, endothelial cells do not express TF activity but do constitutively express TM which accelerates the thrombin-catalysed activation of protein C, thus contributing to the anticoagulant properties of the endothelium. In some pathological situations, when endothelium or monocyte/macrophages are exposed to inflammatory mediators such as LPS or IL-1 β, they can acquire procoagulant properties [2–4]. Indeed, stimulation of these cells by such compounds may alter the antithrombotic properties of the endothelium by inducing the expression of TF and the down-regulation of TM, thereby promoting coagulation and thrombosis [2–4]. Only a few compounds have been shown to reduce the effect of these inflammatory mediators [5–7].

In recent years, polyunsaturated fatty acids such as dietary ω-3 fatty acids have been used to treat and prevent atherosclerosis and, on an experimental basis, to inhibit thrombosis [8–12]. It should be emphasized that these uses are not based upon the premise that thrombosis and atherosclerosis represent essential fatty acid deficiencies, but rather that the polyunsaturated fat may affect these pathological processes through other unknown mechanisms. The purpose of this work was therefore aimed at exploring the action of the principal polyunsaturated fatty acids on TF expression on the surface of endothelial cells and monocyte/macrophages induced by LPS and IL-1β.

Materials and Methods

Chemicals. LPS (lipopolysaccharide from *E. coli* strain: 055:B5), oleic acid (C18:1 ω-9), linoleic acid (C18:2 ω-6), linolenic acid (C18:3 ω-3), palmitic acid (C16:0), endothelial cell growth factor and heparin (sodium salt from porcine intestinal mucosa) were purchased from the Sigma Chemical Co. (France). PPSB, a mixture of blood coagulation factors (factors II, VII, IX and X) was obtained from Intertransfusion (France) and substrate S2222 from Kabi (Sweden). IL-1β was from Tebu (France). Tissue culture reagents were purchased from Boehringer Mannheim (France). All other chemicals were supplied by Prolabo (France).

Cells. HUVEC were isolated and cultured as described previously [13] in 75-cm^2 culture flasks in F12-Ham's medium supplemented with 10% foetal calf serum, penicilin (100 IU/mL), streptomycin (100 μg/mL), glutamine (2 mM), endothelial cell growth factor (30 μg/mL) and heparin (100 μg/mL). Cells were routinely used from the third to the sixth passage. Mononuclear cells were obtained from human heparinized blood as described by Boyum [14]. Cells were plated for 30 min at 37° in 96-well microplates (10^5 cells/well). Non-adherent cells were then harvested and adherent monocyte/macrophage (5×10^3 cells/well) used for the assay.

Determination of tissue factor activity. Procoagulant activity was assayed according to Suprenant and Zuckerman [15]. Briefly, adherent cells were incubated for 18 hr at 37° in M-199 (without phenol red) with endotoxin or IL-1β in the absence or presence of the indicated concentrations of the various fatty acids. These compounds were first solubilized in 60% ethanol in water (v/v) and further diluted in M-199 culture medium + 0.5% bovine serum albumin. The corresponding vehicle was added in the controls. Fatty acids showed no evidence of cytotoxicity at the maximum concentration tested as evidenced by Trypan blue exclusion (not shown). The medium was removed and the wells washed twice with 1 mL of phosphate-buffered saline and incubated for 45 min at 37° with 250 μL of M-199 containing PPSB (0.44 U/mL FVII) and 100 μg/mL of substrate S2222. Optical density (OD) was measured at 405 nm. TF activity was obtained from a standard curve (log [ΔOD_{405}/min] vs log [U/mL]) using serial dilutions of human recombinant TF (Calbiochem, La Jolla, CA, U.S.A.). in M-199 assayed as described above. Undiluted TF was arbitrarily assigned a value of 1 U/mL. TF activity

* Abbreviations: HUVEC, human umbilical vein endothelial cells; IL-1 β, interleukin-1 β; LPS, endotoxin; TF, tissue factor; TM, thrombomodulin.

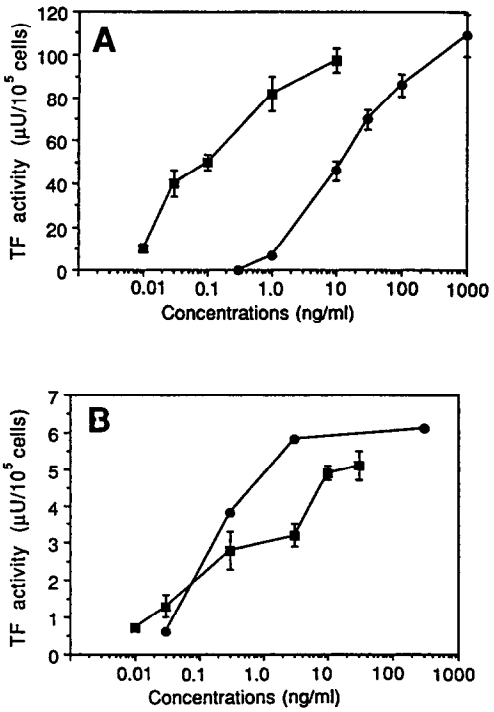

Fig. 1. Effect of LPS and IL-1 β on TF expression in human monocyte/macrophages and HUVEC. Human monocyte/macrophages (A) and HUVEC (B) were incubated with increasing concentrations of LPS (circles) or IL-1β (squares). TF expression was quantified as described in Materials and Methods. Results are expressed as means ± SD (N = 6).

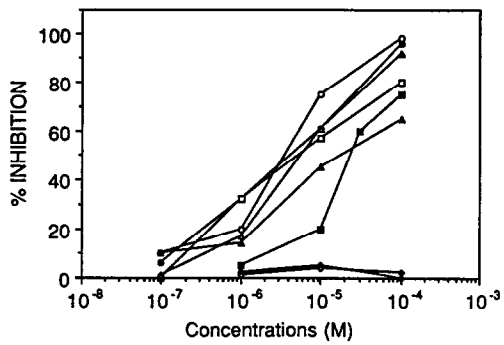

Fig. 2. Effect of various fatty acids on LPS- or IL-1β-induced expression of TF in monocyte/macrophages. Increasing concentrations of oleic acid (●, ○), linoleic acid (■, □), linolenic acid (▲, △) or palmitic acid (◆, ◇) were incubated with human monocyte/macrophages in the presence of LPS (1 μg/mL) (full symbols) or IL-1 β (0.5 ng/mL) (empty symbols). TF expression was determined as described in Materials and Methods. Results are expressed as % inhibition of the control response (N = 6).

was normalized to the cell counts from the same well and expressed as μU of TF/10^5 cells.

Results and Discussion

Unstimulated cells were devoid of TF activity as demonstrated by an insignificant hydrolysis of S2222. However, the addition of LPS or IL-1 β to adherent human monocyte/macrophages or HUVEC resulted in a dose-dependent expression of TF on the cell surface (Fig. 1), therefore confirming previous observations [2–5]. As shown in Fig. 2, oleic acid counteracted LPS- in a dose-dependent manner and IL-1 β-induced TF induction in monocyte/macrophages. The IC_{50} values (concentrations which inhibited 50% of pyrogen-induced TF expression) were of 2.4 ± 0.2 μM and 3.3 ± 0.1 μM with regard to LPS- and IL-1 β-induced TF expression, respectively. Although to a lesser extent, linoleic and linolenic acid also reduced IL-1β-induced TF expression with IC_{50} values shown in Table 1. Palmitic acid remained ineffective up to a concentration of 100 μM. In HUVEC, none of the fatty acids tested altered the pyrogen-induced expression of TF (Table 1), showing that this effect was selective for monocyte/macrophages. This observation is of considerable importance since it shows for the first time that polyunsaturated fatty acids are able to reduce pyrogen-induced TF expression in monocytes. Although relatively few studies have examined the effect of these compounds upon thrombogenesis, a persistent interest in the relationships of polyunsaturated fatty acids to the role of platelets, monocyte/macrophages and endothelium in atherosclerosis and thrombosis demonstrated that incorporation of polyunsaturated fatty acids into the diet significantly reduced experimental thrombosis in

Table 1. Effect of various polyunsaturated fatty acids on pyrogen-induced expression of TF in monocyte/macrophages and HUVEC

	IC_{50} (μM)			
	Monocytes		HUVEC	
Fatty acids	IL-1β	LPS	IL-1β	LPS
Oleic acid	2.4 ± 0.2	3.3 ± 0.1	>10	>10
Linoleic acid	10.3 ± 1.4	3.8 ± 0.7	>10	>10
Linolenic acid	5.1 ± 0.6	3.7 ± 0.1	>10	>10
Palmitic acid	>100	>100	>10	>10

Values are means ± SD (N = 9).

various animal models [8–12]. However, none of these studies were able to establish the exact mechanism of action of these compounds. Of the numerous studies which have examined the effect of feeding polyunsaturated fatty acids to humans, there has been an impressive consistency in the finding that dietary ω-3 fatty acids lower plasma lipid levels but also lead to a reproducible prolongation of bleeding time [16–19], inhibition of platelet aggregation induced by ADP and collagen [16–21] as well as a decrease in platelet retention on glass beads [18]. As already suggested, the mechanisms of these functional alterations can be explained in part by changes in platelet and endothelial cell prostaglandin synthesis induced by alterations in dietary fatty acid composition [22, 23] but our work also shows that ingestion of dietetic polyunsaturated fatty acids may also have a rather profound and specific effect on pyrogen-induced TF expression in monocyte/macrophages. However, more data are needed to determine the exact mechanism of action of such compounds and to characterize further the antithrombotic properties of polyunsaturated fatty acids in humans.

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