

The isoprostane 8-iso-PGF_{2α} stimulates endothelial cells to bind monocytes: differences from thromboxane-mediated endothelial activation¹

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SPECIFIC AIMS

The aim of this study was to investigate whether 8-iso-PGF_{2α}, a nonenzymatic free radical-induced oxidation product of arachidonic acid, activates endothelial cells (EC) to bind monocytes, which is a key initiating event in atherogenesis. Since thromboxane induces leukocyte adhesion to EC and other effects of 8-iso-PGF_{2α} can be blocked by thromboxane receptor antagonists in vitro and in vivo, we wanted to analyze whether 8-iso-PGF_{2α}-induced EC activation differs from that induced by thromboxane.

PRINCIPAL FINDINGS

1. Induction of leukocyte-endothelial interactions and expression of inflammatory adhesion molecules by 8-iso-PGF_{2α} and the thromboxane mimetic U46619

Both 8-iso-PGF_{2α} and the thromboxane mimetic U46619 stimulated EC to bind monocytes in a concentration-dependent manner (**Fig. 1A, B**). Since treatment of EC with a concentration of 10 μM of each of the tested substances gave a reproducible twofold increase in monocyte binding, this concentration was used for all further experiments. Addition of the thromboxane receptor (TP) antagonist SQ29548 (10 μM) abrogated the effects of both 8-iso-PGF_{2α} and U46619 (**Fig. 1C**). Addition of SQ29548 (10 μM) alone had no effect (not shown). To investigate whether the effects of 8-iso-PGF_{2α} and U46619 were specific for induction of monocyte adhesion, effects on neutrophil-endothelial interactions were also examined. U46619, but not 8-iso-PGF_{2α}, stimulated EC to bind neutrophil-like HL-60 cells. Again, addition of SQ29548 abrogated the effect of U46619. On the basis of these results and previous reports that U46619 induced expression of inflammatory adhesion molecules in EC, we compared 8-iso-PGF_{2α} with U46619 on effects on the

expression of VCAM-1 (VCAM-1). Stimulation of EC with U46619 (10 μM, 4 h) strongly induced surface expression of both E-selectin and VCAM-1. However, there was no significant effect of 8-iso-PGF_{2α} on expression of these adhesion molecules. Addition of SQ29548 again blocked the effect of U46619. Treatment of stimulated EC with a VCAM-1 blocking antibody significantly reduced monocyte adhesion to EC treated with U46619 or tumor necrosis factor α (TNF-α), but was without effect on EC treated with 8-iso-PGF_{2α}. These results demonstrate that 8-iso-PGF_{2α} specifically induces monocyte adhesion to EC, whereas the TP agonist U46619 induces monocyte as well as neutrophil adhesion, likely through induction of surface expression of the inflammatory adhesion molecules E-selectin and VCAM-1.

2. Involvement of PKA and PKC in stimulation of monocyte adhesion by 8-iso-PGF_{2α} and U46619

Addition of H89 (25 μM), a PKA inhibitor, blocked 8-iso-PGF_{2α}- but not U46619-induced monocyte adhesion to EC. On the other hand, inhibition of PKC using bisindolylmaleimide I (10 μM) abrogated U46619-induced monocyte binding, but reduced 8-iso-PGF_{2α}-induced monocyte binding by only ~50%. These results demonstrate that induction of monocyte binding by 8-iso-PGF_{2α} involves a PKA-dependent pathway, whereas U46619-induced EC activation is mainly dependent on PKC.

3. Degradation of IκB is not induced by 8-iso-PGF_{2α} and U46619

The transcription factor NF-κB is the major mediator of inflammatory cytokine-dependent EC activation

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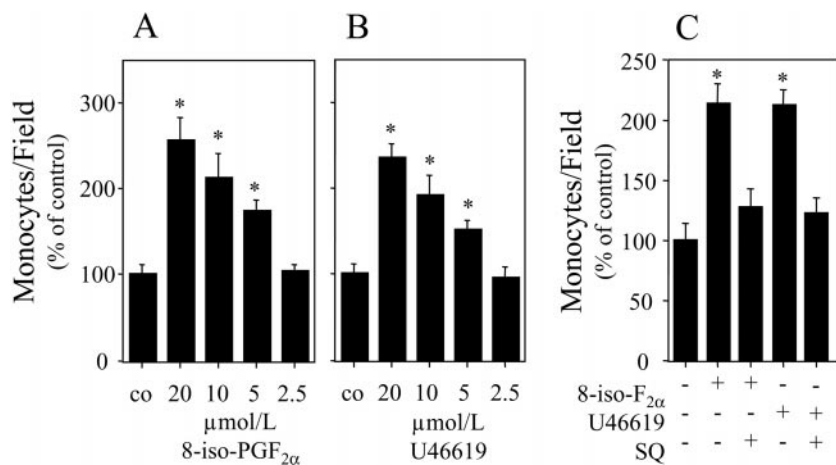


Figure 1. 8-iso-PGF_{2α} and U46619 concentration-dependently stimulate EC to bind monocytes. EC were incubated with indicated concentrations of 8-iso-PGF_{2α} (A) or U46619 (B) for 4 h at 37°C. C) SQ29548 (10 μM) was added together with 8-iso-PGF_{2α} or U46619 (10 μM each). Monocyte adhesion experiments were performed in 48-well plates and performed in triplicate. Statistical analysis was performed using one-way ANOVA. **P* < 0.001, compared with cells treated with medium only (co). Data are expressed as mean ± SE and are representative of 5 independent experiments.

and induces expression of adhesion molecules such as E-selectin and VCAM-1. Classically, NF-κB activation is triggered by phosphorylation and degradation of its inhibitor IκB, which prevents translocation of NF-κB from the cytosol to the nucleus. Measuring phosphorylation and degradation of IκBα by Western blotting, we demonstrate that neither U46619 nor 8-iso-PGF_{2α} altered levels of phosphorylated or total IκBα. When used as positive control, TNF-α induced phosphorylation as well as degradation of IκBα within 10 min and new synthesis of IκBα within 1 h. These results indicate that IκB degradation leading to NF-κB translocation is induced by neither U46619 nor 8-iso-PGF_{2α}; however, *trans*-activation of NF-κB already located in the nucleus, which would result in increased transcriptional activity, cannot be ruled out.

4. Activation of MAP kinases by 8-iso-PGF_{2α} and U46619 and their involvement in stimulation of monocyte adhesion

Another mechanism by which inflammatory genes are up-regulated in EC is activation of MAP kinases. Here we show that phosphorylation of ERK1/2 in EC is induced by both 8-iso-PGF_{2α} and U46619 with similar potency. Maximal activation was reached after 20 min, returning to baseline levels 120 min after stimulation. However, pretreatment of EC for 1 h with an inhibitor for the kinase upstream of ERK, MEK1/2 (PD 98059, 10 μM), blocked stimulation of EC by 8-iso-PGF_{2α} but not by U46619, demonstrating a further difference in mechanisms whereby these two agonists induce monocyte adhesion.

In addition, 8-iso-PGF_{2α} and U46619 both induced phosphorylation of p38^{MAPK}. Pretreatment of EC with an inhibitor of p38^{MAPK}, SB203580 (10 μM), for 1 h abrogated monocyte binding induced by both 8-iso-PGF_{2α} and U46619. These data suggest that the p38^{MAPK} pathway plays an essential role for both 8-iso-PGF_{2α}- and U46619-induced monocyte adhesion to EC.

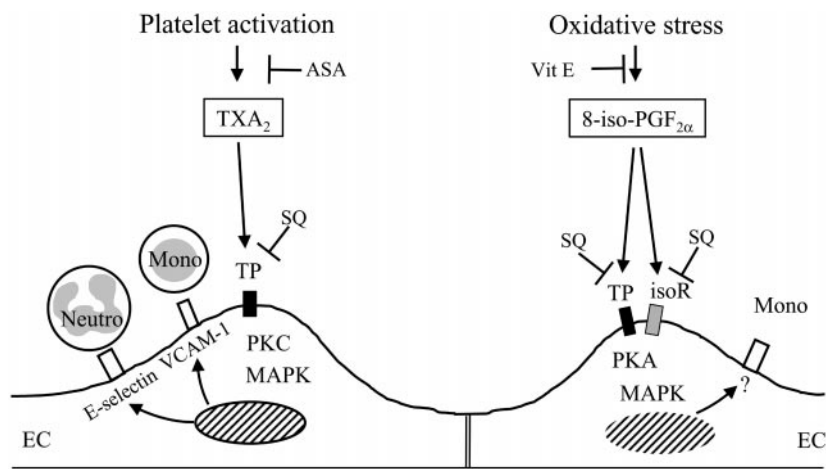
CONCLUSIONS AND SIGNIFICANCE

Isoprostanes are products of nonenzymatic free radical-catalyzed peroxidation of arachidonic acid and were shown to be reliable markers for oxidant stress. Increased levels of circulating isoprostanes were measured in animal models of oxidant injury and in patients with hypercholesterolemia after coronary reperfusion, angioplasty, diabetes mellitus, Alzheimer's disease, hepatorenal syndrome, or scleroderma and in smokers. 8-iso-PGF_{2α} was shown to be present in oxidized low density lipoprotein, in human atherosclerotic lesions, and in apoE null mice, where suppression of isoprostane formation with vitamin E decreased atherosclerosis. Thus, a role for isoprostanes in atherogenesis was suggested.

Various biological effects have been attributed to isoprostanes. 8-iso-PGF_{2α} is a potent vasoconstrictor and was shown to cause bronchoconstriction in rats. On a cellular level, isoprostanes affect platelets, vascular smooth muscle cells, and endothelial cells. These effects of 8-iso-PGF_{2α} could be blocked by a TP antagonist (SQ29548), suggesting that 8-iso-PGF_{2α} acts on TP. Indeed, it has been shown that 8-iso-PGF_{2α} binds to and activates TP. However, evidence for the presence of unique isoprostane receptors comes from receptor binding and ligand displacement studies, suggesting that isoprostanes interact with receptors that are distinct from but closely related to TP. In addition, it has been shown that effects of isoprostanes on the vasculature are mediated via TP *in vivo* and blocking of TP resulted in decreased atherosclerotic lesion formation in apo E null mice. In the latter study, aspirin was ineffective, indicating that eicosanoids other than thromboxane were responsible for activation of TP, which in turn led to increased monocyte adhesion to EC.

Adhesion of monocytes to the endothelium is an initiating event in the development of atherosclerotic lesions. Other forms of oxidized lipids have been demonstrated to specifically induce monocyte-endothelial interactions. Here we show that 8-iso-PGF_{2α} specifically induces monocyte-endothelial interac-

Figure 2. Proposed mechanisms for isoprostane-induced EC activation. In settings of increased oxidative stress, generation and accumulation of isoprostanes occur through oxidation of arachidonic acid. Stimulation of EC by 8-iso-PGF_{2α} via TP or TP-related isoprostane receptors leads to activation of MAP kinase pathways and adhesion of monocytes, involving PKA. In contrast, activation of EC by thromboxane leads to the expression of inflammatory adhesion molecules VCAM-1 and E-selectin, resulting in adhesion of both monocytes and neutrophils.



tions, whereas the TP agonist U46619 also induces neutrophil binding. Although the actions of both 8-iso-PGF_{2α} and U46619 could be inhibited by a TP antagonist, distinct differences in EC activation by 8-iso-PGF_{2α} vs. U46619 could be demonstrated: 1) U46619, but not 8-iso-PGF_{2α}, stimulated EC to bind neutrophils and express E-selectin and VCAM-1; 2) monocyte adhesion induced by U46619 was PKC dependent but PKA independent, whereas 8-iso-PGF_{2α}-induced monocyte adhesion was mainly PKA dependent; 3) 8-iso-PGF_{2α}- but not U46619-induced monocyte adhesion was dependent on MEK-1; 4) both U46619- and 8-iso-PGF_{2α}-induced monocyte adhesion depended on p38^{MAPK}; 5) neither agonist stimulated degradation of IκB.

These results can be explained by two alternative mechanisms. Induction of monocyte adhesion by the isoprostane could be mediated by a unique isoprostane receptor(s) that may be similar to TP with respect to ligand binding but distinct with respect to intracellular signaling. Alternatively, induction of monocyte binding by 8-iso-PGF_{2α} could be mediated by binding to a specific splice variant of the TP. TPα functionally couples to both Gα_q and Gα₁₁ after stimulation with U46619 or 8-iso-PGF_{2α}, whereas TPβ couples to Gα₁₁ and Gα_s. Therefore, the two TP isoforms regulate adenylyl cyclase activity in opposite ways. Whether the differences in EC activation leading to monocyte adhesion by U46619 and 8-iso-PGF_{2α} are due to activation of a specific isoprostane receptor or to differences in the rate of activation of TPα and TPβ leading to differential G-protein coupling cannot be deduced from our data.

It was shown previously that thromboxane-induced adhesion molecule expression in endothelial cells could be blocked by PDTC, a thiol-modifying compound, also known to interfere with activation of NF-κB. It was concluded from these results that the effects of thromboxane were mediated by NF-κB activation. However, PDTC is a rather unspecific inhibitor of NF-κB signaling and interferes with various intracellular signaling cascades. Here we present evidence that at least IκB degradation leading to NF-κB translocation is not induced by thromboxane and therefore is not responsible for the biological effects of thromboxane and 8-iso-PGF_{2α}.

Whereas monocyte adhesion induced by U46619 was mediated by VCAM-1, the adhesion molecules induced by 8-iso-PGF_{2α} are not known.

In conclusion, we have shown for the first time that 8-iso-PGF_{2α} specifically induces monocyte adhesion to endothelial cells and thus is a potentially atherogenic agent whether EC activation is mediated via a specific TP-related isoprostane receptor or by specific occupancy of any of the splice variants of the TP (Fig. 2). These findings are consistent with increased levels of 8-iso-PGF_{2α} found in atherosclerotic lesions and hypercholesteremic patients and with the hypothesis of a TP-mediated, thromboxane-independent mechanism for induction of atherosclerotic lesion development. Our results therefore suggest that 8-iso-PGF_{2α} may play an important role in the development of the atherosclerotic lesion and in other chronic inflammatory diseases. FJ