PGE_2 Cigarette smoke-induced modulates endothelial Tissue Factor: role of EP1 receptor and SIRT1

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Background. Cigarette smoke exposure increases the risk of atherothrombosis, induces the expression of cyclooxygenase-2 (COX-2) and the release COX-2 derived eicosanoids,included Prostaglandin E_2 (PGE_2). Moreover, cigarette smoke up-regulates the expression and activity of Tissue Factor (TF), the main initiator of the coagulation cascade. The relationship between PGE_2 and TF has, however, not been elucidated yet.
Aims. In this study, we analysed the relationship between PGE_2 and TF, and the impact of their regulation on cigarette smoke-induced atherothrombosis.
Methods. The levels of PGE_2 and TF expression and activity in plasma were measured in 20 healthy active smokers (AS) and 46 non-smokers (NS). The expression of PGE_2 and TF was also measured in aorta tissue of mice and in mouse cardiac endothelial cells (MCEC) treated with aqueous extracts of cigarette smoke (TS) plus IL-1β (TS/IL-1β) by EIA assay, real-time PCR and procoagulant activity, using one stage-clotting assay, and/or by Zymuphen MP-TF. Arachidonic acid-induced thrombosis was used to explore the effect of TS/IL-1β in mice.The expression of SIRT1, the NAD^+-dependent protein deacetylase, was measured by Western blotting and by Immunofluorescence.
Results. Higher levels of both PGE_2 and TF were detected in plasma of AS compared to NS. Similar results were obtained in mice and in MCEC treated with TS/IL-1β. A highly significant correlation between PGE_2 and TF activity was observed in both human plasma and mouse tissue. Inhibition of PGE synthase (PGES) activity by CAY10526, or by specific PGES siRNA, markedly diminished both *in vitro* and *in vivo* the amount of TF and the mouse carotid artery thrombosis induced by TS/IL-1b. In contrast, treatment with exogenous PGE_2 increased both TF and arterial mouse thrombosis.
MCEC express three PGE_2 receptors: EP1, EP2 and EP4. In these cells, EP1 antagonists (AH6809 and SC51089) prevented the TF induced by TS/IL-1β. By contrast, an EP1 agonist (17-phenyl-trinor-PGE_2) increased TF. The involvement of other EP receptors was excluded because an EP4 antagonist (GW627368X) and EP2/EP4 agonists (misoprostol, butaprost) did not modify TF. The role of SIRT1 in the regulation of TF was also analysed. Both Sirtinol, an SIRT1 deactivator, and the specific SIRT1 siRNA increased TF expression. By contrast, the SIRT-1 activators (resveratrol and CAY10591) reduced the TF expression and activity induced by both TS/IL-1β and EP1-agonist.
Finally, EP1 agonist or the selective PGES-1 siRNA prevented the inhibition of SIRT1 mediated by PGE_2 or by TS/IL-1β.
Summary/Conclusion. PGE_2 increases the expression and the activity of TF in both mouse carotid arteries and endothelial cells. The phenomenon involves the regulation of the EP1/SIRT-1 pathway. EP1 represents a possible target to prevent thrombotic events.