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PLATELET 12-HYDROXY-5,8,10-HEPTADECATRIENOIC ACID (HHT) STIMULATES PROSTACYCLIN PRODUCTION BY ENDOTHELIAL CELLS. M.J.Stuart, P.D. Sadowitz, and B.N.Y. Setty. SUNY, Health Science Center, Syracuse, N.Y., U.S.A.

Although HHT accounts for approximately one third of the arachidonic acid (AA) metabolites produced by stimulated platelets, no well defined function has been attributed to this platelet product. We report that HHT stimulates prostacyclin production by endothelial cells, and have identified the mechanism for this effect. In human umbilical venous endothelial cells HHT (0.5 and 1μM) stimulated prostacyclin (RIA for $6KPGF_{1\alpha}$) by 32±10% (1SE) and 42±13% (P<0.05 and (0.01). Similar changes were observed when the effect of HHT on exogenous [1-^{14}C] AA metabolism in fetal bovine aortic endothelial cells (FBAECs) was studied. $6KPGF_{1\alpha}$ was stimulated by 25±9% and 30±6% at HHT concs. of 0.5 and 1μM (P<0.05). While prelabelling experiments with [1-^{14}C] AA revealed that HHT (1μM) did not affect the ionophore stimulated release of AA from FBAEC membrane lipids (29521±11837 cpm/well control vs 32458±8811 in HHT treated cells, mean ±1SD) kinetic analyses revealed that HHT affected vascular cyclooxygenase. HHT (1μM) increased Vmax in test microsomes (706±21 pmol/mg/min) when compared to controls (529±20; P<0.02). No effect on Km was observed (6.2±0.3μM control vs. 7.2±0.4 in HHT treated microsomes). The effect of HHT on platelet AA metabolism was next studied. Preincubation of washed platelets with HHT (1μM) did not enhance thrombin (0.2 U/ml) induced plt. TXB_2 (2.27±1.34 pmol/10^6 platelets control vs 2.28±1.62 in HHT treated platelets). In platelets prelabelled with [1-^{14}C] AA, HHT (1μM) also had no effect on AA release post thrombin stimulation (5794±423 cpm per 10^8 platelets control vs. 6135±612 for paired HHT treated cells). Conversion to cyclooxygenase metabolites was also not enhanced (2605±265 vs 2806±332 for test platelets). HHT thus stimulates vascular prostacyclin without a concomitant effect on platelet AA metabolism. Our findings may explain the discrepancies relative to prostacyclin production in atherosclerosis and diabetes. In these disorders while ex vivo production of prostacyclin by vascular tissue is decreased, in vivo production is elevated. HHT may also be an important local modulator of platelet plug formation and could play a protective antithrombotic role by its hitherto unrecognized effect on vascular prostacyclin.

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PROSTACYCLIN (PGI_2) FORMATION IN RELATION TO ANAPHYLATOXIN C5a GENERATION DURING RABBIT ENDOTOXIN SHOCK. M. Rampart (1), H. Bult (1), A.G. Herman (1), P.J. Jose (2) and T.J. Williams (2), University of Antwerp (UIA), Wilrijk, Belgium (1) and Clinical Research Centre, Harrow, UK (2).

Injection of endotoxin (LPS) in animals, a model for gram-negative septic shock, leads to intravascular activation of the complement system, and is one of the few conditions in which 6-oxo-$PGF_{1\alpha}$ and thromboxane (TX) B_2 (non-enzymic metabolites of PGI_2 and TXA_2) can be detected in arterial blood. Previously we reported associations between complement activation, PGI_2 biosynthesis and LPS-induced hypotension in rabbits. As C5a and C5adesArg trigger endothelial PGI_2 formation in vitro, we have now measured the plasma levels of immunoreactive (ir) C5a in relation to generation of PGI_2 and changes in arterial blood pressure in LPS shock. Pentobarbitone anaesthethized rabbits received LPS (E. coli O111:B4, 0.5 mg/kg) or saline via the marginal ear vein. A catheter in the left carotid artery was used to collect blood and to monitor mean arterial blood pressure (MABP). Platelet and leukocyte numbers, haemolytic complement titre (CH50), and plasma ir6-oxo-$PGF_{1\alpha}$, irTXB_2 and irC5a were measured 15 min before and at different times after saline or LPS injection. LPS caused a dose- and time-dependent formation of irC5a in rabbit serum in vitro, predominantly via the classical pathway. LPS also activated complement in vivo, as indicated by about 20 % reduction of CH50 titre (measured after 3h) and a marked increase of arterial irC5a (20-120 ng/ml) in the first 2 to 5 min. After 30 min, irC5a had returned to baseline levels (< 2-5 ng/ml) and remained so up to 3h after injection of LPS. This irC5a peak correlated with a shortlasting initiation of PGI_2 release (from < 20 pg/ml up to 550 pg/ml) and a drop in MABP (from about 95 mmHg to 50 mmHg) 2-5 min after LPS. None of these changes occurred after saline injection.

In conclusion, LPS activates complement in vivo with concomitant formation of C5a. This peptide may trigger - either directly or after phagocyte activation - endothelial PGI_2 biosynthesis, leading to arterial hypotension. This is supported by the suppression of the initial rise of arterial ir6-oxo-$PGF_{1\alpha}$ and hypotension in complement-depleted rabbits. Inhibition of C5a formation or activity may prove to be a meaningful approach to the treatment of septic circulatory shock.

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CULTURED HUMAN MICROVASCULAR ENDOTHELIAL CELLS SYNTHESIZE CYCLOOXYGENASE METABOLITES FROM ARACHIDONIC ACID IN RESPONSE TO LEUKOTRIENES C_4 AND D_4. L.O. Carreras, J. Maclouf, G. Tobelem, and J.P.Caen. Unite 150 INSERM, Hopital Lariboisiere, Paris, France.

Several investigators have demonstrated that endothelial cells have heterogeneous intrinsic properties depending on their vascular origin. In this respect, very limited knowledge exists concerning the production of eicosanoids by human microvascular endothelial cells (HMEC). The aim of this study was to determine: 1) the pattern of the production of cyclooxygenase metabolites by cultured HMEC from omental adipose tissue as compared to the classical study of human umbilical vein endothelial cells (HUVEC); 2) the modification of this metabolism upon leukotrienes (LTs) stimulation. Cultured HMEC produced prostaglandin (PG) E_2, PGF_2, 6-keto-PGF_1, and PGD_2 (measured by enzymoimmunoassay). In basal conditions, PGD_2 was the main product released in the supernatant. Upon stimulation with thrombin, arachidonic acid and calcium ionophore A23187, a marked increase in the production of PGE_2, PGF_2, and 6-keto-PGF_1, was observed; these results were quite different from HUVEC. In contrast, PGD_2 remained unchanged under our experimental conditions and thromboxane B_2 was always undetectable. In all cases, the release of PGE_2 and PGF_2, was higher than that of 6-keto-PGF_1. A considerable amount of the metabolites produced remained cell-associated. The total production (release + cell bound) of cyclooxygenase products was stimulated by LTC_4 and LTD_4 in a dose-dependent manner (10^{-9} to 10^{-6} M). The production of PGD_2 was unchanged. LTC_4 and LTD_4 were almost equally potent, but LTB_4 was unable to stimulate PG synthesis (n=4). The production of metabolites induced by 1 uM LTC_4 or LTD_4 was even higher than that obtained in the presence of high concentrations of thrombin (5 U/ml). This contrasted with the more pronounced stimulation of thrombin on HUVEC as compared to LTs. In the kinetic studies (n=2) we have observed a slow time-course of release of PGE_2 and 6-keto-PGF_1 into the supernatant of LTs-stimulated HMEC (half-maximal formation at 14-15 min). The stimulatory activity of LTC_4 and LTD_4 on the production of vasoactive cyclooxygenase metabolites by HMEC could be relevant in inflammatory processes.

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INCREASED IN VIVO FORMATION OF THROMBOXANE AND PROSTACYCLIN IN HUMANS AFTER AORTIC REPLACEMENT WITH SYNTHETIC GRAFTS. J.Swedenborg, C.Greén, J.Lewin and O.Vesterquist. Departments of Clinical Chemistry and Surgery, Karolinska Hospital, Stockholm, Sweden

Replacement of arteries with synthetic grafts causes activation of both plasma coagulation and platelets. In order to measure platelet activation the in vivo production of thromboxane A_2 (TxA_2) and prostacyclin (PGI_2) were measured in patients following graft replacement of the abdominal aorta for aneurysmal disease.

Specific methods based on gas chromatography-mass spectrometry using tetra-deuterated internal standards/carriers were used to measure the urinary excretion of 2,3-dinor-TxB_2 and 2,3-dinor-6-keto $PGF_{1\alpha}$, the two major urinary metabolites of TxA_2 and PGI_2. The excretion of the metabolites increased ten-fold and forty-fold respectively on the first postoperative day and remained elevated up till 10 days postoperatively. In patients undergoing cholecystectomy only minor changes of shorter duration were seen. A marked decrease in platelet count occurred concomitanly with the increase in the urinary metabolites. Platelet counts returned to normal or supernormal values after 10 days when the excretion of 2,3-dinor TxB_2 had returned to normal values.

It is concluded that synthetic grafts cause prolonged increase in the in vivo formation of TxA_2 and PGI_2 concomitantly with a decrease in platelet count. The reason for the increased TxA_2 formation may be platelet interaction with the foreign surface but the increase of PGI_2 is unexplained. The latter increase could be part of a vascular defense against the induced thrombotic activity.