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5-LIPOOXYGENASE ACTIVITY IN THE HUMAN VESSEL WALL

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5-lipooxygenase products have been identified from a variety of cells and may play a role in the progression of atherosclerosis and in its clinical manifestations (spasm, thrombosis). We investigated whether human vascular fragments, freshly obtained at surgery, are able to produce leukotriene (LT) B4, a definite end product of 5-lipooxygenase, provided with biological activity. Fragments obtained from human saphenous veins (n=21) or aorta (fibrous plaques, n=15, atheromas, n=16) were incubated in buffer at 37°C with mechanical agitation sequentially in the absence (15 min) and in the presence (15 min) of 10 μM calcium ionophore A-23187. At the end of each incubation, the buffer was sampled to be assayed by a specific radioimmunassay (RIA) for LTB4 (sensitivity 4.3±0.9 pg). Validation of the assay was performed by comparison with a chemotactic bioassay in Boyden chambers, by interpolation of a standard curve evaluating the chemotactic response of neutrophils to a standard LTB4 preparation. RIA resulted the only practicable method to detect concentrations lower than 2.5 ng/ml, compared both to bioassay and to HPLC, all three performed in the incubation media from 8 vascular fragments. Incubations were also performed in a chamber with selective exposure of the endothelial surface in order to detect possible production of LTB4 on the luminal site of the vessel. Both unstimulated and ionophore-stimulated LTB4 were higher ($P < 0.01$) in atheromas (2.7±1.2 and 6.3±1.8) than in fibrous plaques (0.51±0.22 and 1.19±0.38) or saphenous veins (0.74±0.34 and 3.07±1.39) (ng/g wet weight, mean±SD). Detectable spontaneous and stimulated LTB4 productions were also found in the incubation media of the chamber with atheromas (40±14 and 324±85 pg/cm^2 area, respectively). Histology of the fragments confirmed a higher cellularity (macrophages, atherocytes) in atheromas as compared to fibrous plaques and veins. The human vascular wall is a definite site of 5-lipooxygenase activity, possibly arising from white cell infiltration. LTB4 production, able to reach the inner vessel surface and the blood stream, is a possible factor in the progression of the lesion by increasing vascular permeability or recruiting white blood cells.

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PROCOAGULANT ACTIVITY OF PLATELET LIPOXYGENASE PRODUCTS.

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Arachidonic acid is metabolised by cyclo-oxygenase and lipoxygenase enzymes in platelets. Cyclo-oxygenase produces the highly pro-aggregatory thromboxane A_2, but the physiological significance of the lipoxygenase pathway in platelets remains uncertain. Arachidonic acid can also be converted to biologically active peroxides by free radical induced autoxidation, and previous studies have shown that such products generate large amounts of thrombin in platelet-free plasma, via their interaction with plasma lipoproteins.

In the present study, we have investigated the procoagulant activities of platelet lipoxygenase products and compared them with autoxidised arachidonic acid. Platelet concentrates were incubated with arachidonic acid and indomethacin for 30 minutes and the products extracted with ethyl acetate. After drying down, reconstitution in ethanol and partitioning with petrolelm ether to remove unchanged acid, contaminating platelet phospholipid was removed by TLC. By inclusion of ^{14}C-arachidonic acid, average conversion was estimated as 17% in six experiments.

The products promoted the generation of large amounts of thrombin in platelet-free plasma; the peak thrombin averaged 23.1 iu/ml with three different batches. The activity was similar to that of a procoagulant phospholipid (PL) but, unlike PL, the lipoxygenase products did not shorten the kaolin recalcification time, confirming the absence of platelet PL contamination, and were virtually inactive in lipoprotein-free plasma. The activity of the lipoxygenase products was therefore similar to that of autoxidised arachidonic acid in its requirement for plasma lipoproteins. Further TLC separation of the products showed that the activity was associated with a minor component of the mixture which was active at plasma concentrations below 10 μg/ml.

These results suggest a possible role for platelet lipoxygenase products in the coagulation system and provide a novel link between platelets, lipoproteins and coagulation which could be important in the pathogenesis of atherosclerosis.

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THE MITOGENIC EFFECT OF 15-HETE ON ENDOTHELIAL CELLS IS MEDIATED VIA DIGLYCERIDE KINASE INHIBITION.

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We have previously demonstrated that 15-HETE, a mitogen for fetal bovine aortic endothelial cells (FBAECs), caused an accumulation of diglyceride (DG). Stimulation of DNA synthesis by OAG (a synthetic DG analog) suggested that this mitogenic effect was mediated via elevation of cellular DG levels. In the present study we have delineated the mechanism by which 15-HETE causes DG accumulation by evaluating the effects of 15-HETE on the phosphatidylinositol (PI) cycle. In [^{3}H]inositol labeled cells, 15-HETE caused a decrease in PI content under both basal conditions (27%) and following A23187 stimulation (23%). The accumulation of DG and the decrease in PI suggested activation of phosphilipase C. However, no effect of 15-HETE was found on endothelial cell phospholipase C. A modulatory effect of 15-HETE on PI resynthesis was next evaluated using [^{3}H] inositol. In 5 experiments, 15-HETE (30μM) inhibited the synthesis of PI in both unstimulated (51±15%, 1SD, $P<0.001$) and A23187 stimulated cells (80±19%, $P<0.001$). DG accumulation and inhibition of PI synthesis suggested a 15-HETE effect on the conversion of DG to phosphatidic acid (PA) via DG kinase. Using endothelial cell membranes as a source of this enzyme, formation of PA was nonlinear with time, suggesting an interference by PA phosphatase on PA formation. Human red cell membranes were therefore used in lieu of FBAECs as a source of DG kinase. In this system, production of PA was inhibited by 15-HETE in a concentration-dependent manner (IC50 of 22μM). This is the first report of the presence of a DG kinase inhibitor of biological origin. Our studies also delineate the mechanism by which 15-HETE exerts its mitogenic effect on endothelial cells.

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EFFECTS OF 13-HODE AND HETEs ON TUMOR CELL/ENDOTHELIAL CELL INTERACTIONS.

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We and others reported that endothelial cells (ECs) convert linoleic acid into 13-hydroxyoctadecadienoic acid (13-HODE) under basal conditions, and arachidonic acid into 15-hydroxyeicosatetraenoic acid (15-HETE) following stimulation (1,2). We also reported that lipoxygenase metabolism influenced platelet (PLT) interactions with ECs, tumor cells (TCs) and extracellular matrix (BM) (1,3,4). Thus, we performed studies to determine i) if TCs also produce 13-HODE and HETEs, and ii) the effect of TC and EC 13-HODE and HETEs synthesis on TC/EC adhesion. We measured i) the ratios of 13-HODE:HETE in 5TC lines, under basal and stimulated conditions, in metastatic and non-metastatic TCs of the same cell line, and TCs treated with salicylate (SAL) or dipyridamole (DIP), and ii) their relationships with TC adhesion to ECs and BM. 13-HODE and HETEs were assayed by HPLC. TC adhesion was assayed as the # radiolabelled TCs adherent to ECs or BM. cAMP was assayed by RIA. Under basal conditions, TCs produced 13-HODE and HETEs, the intracellular ratio of which markedly affected their adhesivity; e.g. the least adhesive TC (U87MG glioblastoma) produced 21Xs more 13-HODE than HETE's, while a more adhesive TC (A549, adenocarcinoma) produced 4Xs more HETEs than 13-HODE. Non-metastatic TCs preferentially produced 13-HODE while metastatic TCs of the same cell line, produced HETEs. Stimulation of TCs or ECs decreased 13-HODE, and increased HETE synthesis and TC/EC adhesion. Inhibiting intracellular 13-HODE synthesis in either TCs or EC (SAL R_X) enhanced TC/EC and TC/BM adhesion. Enhancing 13-HODE synthesis by elevating cAMP (DIP R_X) inhibited TC/EC and TC/BM adhesion. We conclude that 1) in vitro TCs produce 13-HODE and HETEs, 2) the ratio of 13-HODE:HETEs in TCs and ECs affects their adhesivity; and 3) the ratio of intracellular 13-HODE:HETEs depends upon cAMP. This suggests that 13-HODE:HETE ratios in TCs and ECs influence the adhesion process in the pathogenesis of thrombosis and metastasis in vivo. (1) Buchanan et al, JBC 30:1985. (2) Hopkins et al, JBC 29:1984. (3) Bastida et al, Int. J. Canc. 1987. (4) Buchanan et al, Prost. Leuk. Med., 1986.