688

48740 RP INHIBITS PLATELET SHAPE CHANGE INDUCED BY PAF-ACETHER IN VITRO. G. Jung (1) and P. Sedivy (2). (1) Centre Hospitalier Laboratoire Hématologie 68051 Mulhouse, France, (2) Rhône-Poulenc Santé, 94403 Vitry-sur-Seine, France.

Platelet shape change (PSC) is the first measurable physiological response to platelet pro-aggregant agonists such as PAF-acether (PAF) (1). The laser thromborheometer (2) was used to assess the PSC (detected as decrease in light scattering as % of maximal velocity) and the platelet aggregation (detected as increase in the light transmission as % of maximal velocity and maximal amplitude) in citrated platelet-rich plasma (cPRP) (1 volume 3.8 % sodium citrate + 9 volumes blood) from 12 volunteers of either sex. PAF concentrations ranging from 0.1 to 1 µM induced PSC (range of velocities : 13.2-69.8 % of maximal velocity, n = 12) and platelet aggregation (range of velocities : 9.3-22.2 % of maximal velocity and maximum amplitude of aggregation always superior to 20 %, n = 12). These effects were inhibited by 48740 R.P., 3-(3-pyridyl)-1H,3H pyrrolo [1,2-c]thiazole-7-carboxamide (100-200 µM) a selective and competitive PAF-antagonist (3). For instance, 100 µM 48740 RP decreased by 76.4 ± 3.5 (mean ± S.E.M., n=3) PSC evoked by 0.1 µM of PAF. Furthermore, 48740 RP was also able to reduce PSC in heparinized platelet-rich plasma (hPRP). The antagonist effects of 48740 RP could be overcome by increasing PAF concentrations in the cPRP and hPRP. During this study, 6 volunteers were found, in whom even concentrations of PAF up to 10.5 µM failed to induce an amplitude of aggregation in cPRP greater than 20 % whereas PSC was in the range of 19.1-79.4 % of maximal velocity. These responses to PAF which were antagonized by 48740 RP were not due to an alteration in plasma properties ; thus, they are proposed to result from undetermined changes occuring at the level of the platelet (e.g.) decreased number of PAF-operational receptors or alteration of the excitation-response pathways). In conclusion, 48740 RP is an antagonist of PAF-evoked aggregation and PSC in cPRP from volunteers responding either normally or poorly to PAF.

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689

SELECTIVE ANTAGONISTS OF PAF: INTERFERENCE WITH PLATELET FUNCTION AND EFFECT ON THROMBOGENESIS INDUCED BY SUBENDOTHELIUM.
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Platelet activating factor (PAF) is a very potent excitatory agonist of blood platelets but the physiological importance of this mediator in platelet thrombus formation is not known. We investigated the effect of two chemically unrelated selective inhibitors of PAF-induced platelet aggregation on thrombogenesis induced by rabbit aorta subendothelium (SE) using an ex vivo perfusion system.
Ro 19-3704 is a highly potent inhibitor structurally related to PAF. This compound inhibits PAF-induced aggregation of rabbit platelets in platelet rich plasma in vitro competitively. Against 4 nM PAF, a concentration resulting in submaximal platelet aggregation velocity, the IC_{50} was 70 nM. Inhibition was highly selective for PAF-induced aggregation, since aggregation induced by collagen (HORM, 5 µg/ml), ADP (1 µM) or thrombin (0.4 U/ml) was not inhibited even at a concentration as high as 10 µM. Brotizolam, a triazolobenzodiazepine reported to be a selective inhibitor of PAF-induced platelet activation, had in our system an IC_{50} of 200 nM. The selective benzodiazepine antagonist Ro 15-1788 was without effect on inhibition of PAF-induced platelet activation by brotizolam.
Ro 19-3704 was given intravenously to rabbits as a bolus of 0.2 mg/kg followed by constant infusion of 0.02 mg/kg/min. This dosage provoked ex vivo a constant right shift ratio of the dose response curve for PAF-induced aggregation (RSR[PAF]) by a factor of 25 to 35. Brotizolam was given orally at a dose of 100 mg/kg together with 300 mg/kg of Ro 15-1788 (to antagonize the central effects) 90 minutes before starting the perfusion experiment, resulting in a RSR[PAF] of 35 to 135. ADP induced platelet aggregation was not impaired by either compound. SE was exposed to the non-anticoagulated blood withdrawn from the carotid artery for 3 min at 2600 s^{-1} and for 20 min at 200 s^{-1} shear rate. Quantitative morphometric evaluation showed that SE coverage by platelets and by fibrin, thrombus area and thrombus height were all unchanged by the PAF antagonists at low and at high shear rates despite a very substantial inhibition of PAF-induced platelet aggregation. Therefore a major role of PAF in SE-induced thrombogenesis seems unlikely.

PLATELET SECRETION AND SECRETION PRODUCTS

690

A PLASMA FACTOR FROM A PATIENT WITH A BLEEDING TENDENCY CAUSES PLATELET SECRETION IN THE ABSENCE OF EXTRACELLULAR CALCIUM
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A woman with a history of bruising and bleeding but with a normal platelet count and normal clotting factors, had platelets that appeared grey when stained and viewed under the microscope. Unlike the grey-platelet syndrome, the abnormality was only evident when the blood had been collected into EDTA and not when citrate or heparin was used as anticoagulant. When we examined the EDTA-blood further we found that large quantities of beta-thromboglobulin and serotonin (5HT) were present in the plasma with only small quantities in the platelets. The reverse was the case for blood collected into citrate or heparin. LDH (a cytoplasmic marker) levels in EDTA-plasma were not raised.

Platelet-rich plasma (PRP) was prepared from blood collected into heparin and labelled with ^{14}C-5HT. Incubating the PRP with EDTA (4mM) or EGTA (3mM, sufficient to chelate all the plasma calcium but not the magnesium) caused extensive release of ^{14}C-5HT from the platelets. When Ca^{++} was removed by passing the PRP through an ion-exchange resin, extensive ^{14}C-5HT release also occurred. The release reaction induced by exposing the platelets to a low-Ca^{++} environment could be prevented by agents that increase cAMP levels.

In a series of cross-over experiments, we discovered that the platelet secretion that occurred on removing Ca^{++} was caused by a plasma factor. Platelets from a healthy donor which had been labelled with ^{14}C-5HT were resuspended in the patient's heparinised plasma. Incubating the platelet suspension with EGTA resulted in extensive release of ^{14}C-5HT. Heparinised plasma from the patient was also passed through a column of Protein A-Sepharose to remove the immunoglobulin fraction. EGTA-challenge of control platelets resuspended in the eluted plasma did not cause any release of ^{14}C-5HT, suggesting that the plasma factor responsible may be an immunoglobulin.

We have yet to discover whether this new abnormality (that on initial investigation could be confused with the grey-platelet syndrome) has any relevance to the in vivo bleeding situation in the patient in whom it was discovered.

691

PROTEASE NEXIN II IN HUMAN PLATELETS. W.E. Van Nostrand and D.D. Cunningham. Department of Microbiology and Molecular Genetics, University of California, Irvine, CA, USA.

Normal human fibroblasts secrete a protein into the culture medium named protease nexin II (PN II), which forms sodium dodecyl sulfate (SDS)-stable complexes with epidermal growth factor binding protein (EGF BP). These complexes then bind back to the same cells and are rapidly internalized and degraded. We recently purified PN II to apparent homogeneity and showed that it is a single chain polypeptide with an estimated molecular weight of 106 KDa. Other interesting properties of this protein include its ability to bind heparin and its stability to treatment with SDS or pH 1.5. In addition to EGF BP, PN II will also complex the gamma subunit of 7S nerve growth factor (NGF-gamma) and trypsin. Here we show that human platelets contain a protein which possesses the same properties as does PN II from cultured human fibroblasts. This platelet PN II (PL-PN II) is also a 106 KDa single chain polypeptide which can complex EGF BP, NGF-gamma and trypsin. PL-PN II also possesses the unusual stability to treatment with SDS or pH 1.5. In addition, both PN II and PL-PN II exhibit the same affinity for binding to heparin-Sepharose. Furthermore, rabbit polyclonal antiserum raised against PN II recognized PL-PN II on Western blot analysis demonstrating that both proteins are immunologically related. Treatment of fresh unactivated platelets with epinephrine or thrombin resulted in a release of PL-PN II into the supernatant suggesting that PL-PN II is stored in the platelet alpha granules.