0627

08:45 h

FOLLOWING MILD CAROTID ENDOTHELIAL INJURY, PLATELET-ARTERIAL WALL INTERACTION IS IMPAIRED IN PIGS WITH VON WILLEBRAND'S DISEASE. V. Fuster, M.K. Dewanjee, M.P. Kaye, D.N. Fass, J.G. White, E.J.W. Bowie. Mayo Clinic, Rochester, MN and the University of Minnesota, Minneapolis, MN, U.S.A.

Platelet-arterial wall interaction appears to be important in the genesis of atherosclerosis. Pigs with homozygous von Willebrand's disease (vWd) appear resistant to atherosclerosis. We investigated whether there is impairment in platelet-arterial wall interaction in porcine vWd. Superficial endothelial injury was produced in a 4 cm segment of carotid artery by drying for 3 minutes in a stream of air which entered one end (100 ml/min) and exited through a small hole in the other end. This procedure was performed in 9 normal and 6 vWd pigs 24 hours after the I.V. infusion of III-Indium-labeled platelets. Carotid blood flow was re-established and the pigs were sacrificed 24 hours later and the carotids fixed in glutaraldehyde.

The ratio of radioactivity (cpm/g) of injured/noninjured carotid arteries was markedly decreased in the vWd pigs. In addition, transmission and scanning electron microscopy confirmed superficial endothelial damage in all injured segments, but only significant platelet deposition in the normal pig injured segments.	Normal (n=9)	vWd (n=6)
	53	16
	49	14
	45	5
	34	4
	30	3
	30	2
	25	
	21	
	6	

After a mild endothelial injury, this "in vivo" evidence of impaired platelet-arterial wall interaction in vWd may be related to the resistance to atherosclerosis in these animals.

0628

09:00 h

COMPARISON OF THE EFFECTS OF ASPIRIN AND HEPARIN ON PLATELET ACCUMULATION ON THE INJURED NEOINTIMA OF RABBIT AORTAE. H.M. Groves, R.L. Kinlough-Rathbone and J.F. Mustard. McMaster University, Hamilton, Ontario, CANADA.

We have previously shown in rabbits that treatment with aspirin (ASA) in doses that inhibit PGI_2 production by the aorta does not cause platelet accumulation on the endothelium or cause increased accumulation on the subendothelium when the endothelium has been removed with a balloon catheter. We have also shown that, in contrast to the single layer of platelets that adheres to the subendothelium, extensive microthrombus formation occurs on the surface of the injured neointima. The objective of the present experiments was to compare the effects of ASA and heparin on platelet accumulation on the injured neointima. The endothelium was removed from rabbit aortae with a balloon catheter and at 7 days, when a substantial neointima had formed and was essentially non-reactive to the circulating platelets, the aortae were again injured by the passage of a balloon catheter. Platelet accumulation (measured with ^{51}Cr-labeled platelets) was approx. 50,000 per mm^2 whereas on the uninjured neointima at 7 days it was 1,200 per mm^2. Administration of ASA (in a dose that inhibits platelet function and PGI_2 production by normal vessels, 100 mg/kg I.V.) 10 min before the neointima was injured did not cause a significant increase or decrease in the number of platelets that accumulated on the injured neointima (53,200 ± 11,200 platelets per mm^2 without ASA; 48,100 ± 7,900 platelets per mm^2 for ASA-treated rabbits). In contrast to the effects of ASA, heparin (500 U/kg), as described previously, caused a significant reduction in the number of platelets that accumulated on the injured neointima (50,000 ± 8,800 platelets per mm^2 without heparin; 25,400 ± 5,500 platelets per mm^2 for heparin-treated animals ($p<0.05$). The results from these experiments indicate that ASA inhibition of platelet function and PGI_2 production by the vessel wall does not change platelet accumulation that occurs upon injury of the neointima of a large artery. However, the results with heparin indicate that heparin changes the extent of platelet accumulation on the injured neointima.

0629

09:15 h

EFFECT OF SELECTIVE INHIBITION OF PLATELET THROMBOXANE ON PLATELET-VESSEL WALL INTERACTION IN VIVO. Y.C. Chen, K.K. Wu, E.R. Hall, D.L. Venton and G.C. Le Breton. Department of Medicine, Rush University and Department of Pharmacology, University of Illinois, Chicago, IL, U.S.A.

It is well recognized that thromboxane A_2(TXA_2) plays an important role in platelet reactivity. To determine the role of TXA_2 in platelet-vessel wall (P-V) interaction, the effect of 1-benzylimidazole (1-BI), a specific inhibitor of thromboxane synthetase, and 13-azaprostanoic acid (APA), a TXA_2 antagonist, on platelet thrombus formation was evaluated in vivo in NZW male rabbits using the autologous indium-111(^{111}In) labeled platelet technique. Rabbits were treated with intravenous 1-BI or APA or vehicles. After injection of autologous ^{111}In-platelets, de-endothelialization of the abdominal aorta was created by a balloon catheter technique. At 3 hrs, blood samples were obtained and the animals were sacrificed. The aortae were removed and the injured and uninjured segments were dissected. Radioactivity counts and dry weight of the tissues and blood were determined. The vascular radioactivity counts were converted to platelet numbers by using a standard linear calibration curve. As small numbers of platelets adhered to normal vessel wall nonspecifically, this number was subtracted to obtain specific platelet accumulation at the injured sites. 1-BI at 10mg/kg reduced the specific platelet accumulation significantly (n=5, $12.3 \pm S.D.1.5 \times 10^6$ pl/gm tissue; $p<0.01$) when compared with the controls (n=10, $33.0 \pm 5.1 \times 10^6$ pl/gm tissue). Platelet accumulation was further reduced by increasing the dosage to 30mg/kg. By contrast, APA injection (10mg/kg) had no significant effect. However, when APA was given by constant infusion at 250μg/kg/min 1 hr prior to injury, the APA-treated animals had an 80% reduction of platelet accumulation relative to controls. These findings indicate that TXA_2 plays an important role in P-V interaction and specific inhibition of TXA_2 appears to be efficacious in eliminating platelet thrombus formation.

0630

09:45 h

ENDOTOXIN-MEDIATED ENDOTHELIAL INJURY IN VITRO. J. M. Harlan, L. A. Harker, G. E. Striker, R. B. Counts, University of Washington and Puget Sound Blood Center, Seattle, Washington, USA.

Endotoxin (ET) infusion into animals produces endothelial cell (EC) injury. It is not clear whether EC injury is produced by ET directly or by an ET-generated mediator, or whether ET-mediated EC injury also occurs in man and non-human primates. To answer these questions we examined the effects of ET on human umbilical vein (HUVEC) and bovine aortic (BAEC) endothelial cells in culture. ET (100 ug/ml) + complement + neutrophils produced no significant HUVEC ^{51}Cr-release or cell detachment at 4 hours. HUVEC proliferation by direct cell count, angiotensin converting enzyme activity by labeled substrate conversion, VIII-Ag release by RIA and fibronectin release by ELISA were not affected by ET (100 ug/ml). ET alone did not induce significant release of PGI_2 measured by bioassay or 6-keto-$PGF_{1\alpha}$ measured by RIA. In contrast to the lack of effect of ET on HUVEC, ET produced a time (3 hours: 22.0 ± 4.0%; 6 hours: 40.0 ± 6.0%; 24 hours: 71.0 ± 5.0%) and dose (100 pg/ml: 25.4 ± 2.5%; 1 ug/ml: 83.0± 4.0%; 10 ug/ml: 92.0 ± 3.0%) dependent BAEC detachment which was initially sublethal without ^{51}Cr-release or trypan blue dye uptake (40.6 ± 6.0% detachment, <1% ^{51}Cr-release at 6 hours with ET 10 ug/ml). BAEC detachment was seen with all ET preparations including lipid A. ET-mediated BAEC detachment was inhibited by incubation at 4° but not by indomethacin, methyl prednisolone, or chlorpromazine and was not dependent on serum or complement. Examination of ET-detached BAEC by SDS-PAGE after ^{125}I-surface labeling or by immunofluorescence using anti-fibronectin antibody revealed complete loss of cell surface fibronectin. ET-mediated detachment was also seen with bovine mesenteric EC but not with bovine aortic smooth muscle cells or monkey aortic EC. These studies demonstrate the importance of studying EC from appropriate species and vascular sites. Although ET has no cytotoxic effect on HUVEC, it does induce selective, sublethal BAEC detachment associated with loss of cell surface fibronectin. If a similar effect occurs in human adult arterial or microvascular EC, it would have profound pathophysiologic effects accounting for the permeability changes and thrombosis seen in sepsis in man.