700

NEUTRALIZING EFFECT OF PLATELET FACTOR 4 OR HISTIGINE-RICH GLYCOPROTEIN AGAINST LOW MOLECULAR WEIGHT HEPARIN AND UNFRACTIONATED HEPARIN. K. Takahashi, M. Niwa and N. Sakuragawa. Central Clinical Laboratory, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Purpose: Low molecular weight(LMW) heparin shows stronger anti-factor Xa(F-Xa) and weaker anti-thrombin(TH) activities compared with unfractionated(UF) heparin, and shows less bleeding tendency in the cases of clinical use. Platelet factor 4(Pf-4) and histidine-rich glycoprotein(HRG) neutralize heparin. We investigated on the heparin neutralizing effects of them to both kinds of heparin.
Materials and methods: LMW heparin(Kabi and Pharmuka) and UF heparin(Novo) were used. Antithrombin III(AT-III), HRG(human origin) and pf-4(bovine origin) were purified by our methods. TH(Green-Cross) and F-Xa(Sigma) were used. Reaction mixtures for anti-TH or anti-F-Xa were as follows: 1 vol of AT-III(0.1 U/ml)+ 1 vol of heparin(10 ug/ml)+1 vol of pf-4 or HRG(varied)→incubated for 5 min→+1 vol of TH(5 U/ml) or F-Xa(7 nKat/ml)→incubated for 5 min→ + S-2238 or S-2222→ recorded at 405 nm.
Results: (1) Pf-4 showed the equivalent anti-TH effect on both kinds of heparin, and 3 ug of pf-4 neutralized 1 ug of heparin. On F-Xa neutralizing effect, 13 ug of pf-4 neutralized 1 ug of UF heparin, but could not neutralize LMW heparin. (2) HRG showed the same results on anti-TH effect of both kinds of heparin, but could not neutralize the anti-F-Xa effect of LMW heparin on the same amount of HRG which neutralized that of UF heparin.
Conclusion: Anti-F-Xa effect of LMW heparin could not be easily neutralized by pf-4 or HRG compared with that of UF heparin.

701

INTERACTION OF PLATELET FACTOR 4 WITH HEPATOCYTES AND ITS POSSIBLE SIGNIFICANCE IN HEMOSTASIS. B. Rucinski, G.J. Stewart, G. Boden and S. Niewiarowski. Thrombosis Research Center, Department of Physiology, Temple University School of Medicine, Philadelphia, PA 19140.

Previous experiments have demonstrated the rapid clearance of human platelet factor 4 (PF4) from rabbit and rat blood, its accumulation in the liver and its elimination of PF4 degradation products with urine. Injection of heparin resulted in a rapid loss of ^{125}I-PF4-radioactivity from the liver (Rucinski et al Amer. J. Physiol. 251, H800, 1986). Current experiments demonstrate the uptake of human ^{125}I-PF4 by hepatocytes reaching maximum at 180 min. This uptake is 2-3 times greater at 37°C than at 4°C. At 37°C degradation of ^{125}I-PF4 by hepatocytes was also observed as indicated by the increase of ^{125}I-PF4 radioactivity soluble in 6% trichloracetic acid. By contrast, no uptake of ^{125}I-βTG was observed. Autoradiography demonstrated association of ^{125}I-PF4 with hepatocytes membranes while after longer incubation (20-60 min) radioactivity was also localized in endosomes. The heparin inhibited binding and uptake of PF4 by hepatocytes; accordingly the injection of heparin to or into rabbits within 10 min resulted in a rapid urinary clearance of ^{125}I-PF4 and the injection of PF4 resulted in a rapid urinary clearance of ^{3}H-heparin. We propose that 1) PF4 released to the circulating blood by activated platelets is bound to the surface of hepatocytes and that it is further processed by these cells over time; 2) the presence of PF4 in the liver may contribute to the regulation of hemostasis by neutralizing anticoagulant activity of endogenous heparin-like molecules; 3) in clinical situations PF4 may enhance clearance of injected heparin by accelerating its urinary excretion.

702

IN VIVO INTERACTION BETWEEN HUMAN PLATELET FACTOR 4 (PF4) AND PROTAMINE SULPHATE (PS) IN THE PRESENCE OF DIFFERENT GLYCOSAMINOGLYCANS (GAGs). G. Cella (1), M. Prosdocimi (2), A.A. Sasahara (1). VA West Roxbury and Harvard Medical School, Boston, MA 02115, U.S.A. and the 2nd Chair of Medicine, University of Padua Medical School, 35100 Padua, Italy (1) and Fidia Research Laboratories, 35031 Abano Terme, PD, Italy (2).

Anti-heparin substances, like PF4 or PS, have been studied largely in reference to their ability to neutralize the anticoagulant activity of heparin. On the other hand, few data are available concerning the relationship between GAGs and anti-heparin proteins clearance. We studied the action of PS on human PF4 kinetics in anesthetized rabbits pre-treated with heparin (H, 1000 I.U.), heparan sulphate (HS, 30 mg) and dermatan sulphate (DS, 30 mg). PF4 (given at a dose of 45 µg/kg) disappearance reflected its different affinity for the GAGs, with the following half lives (min): control 2.09±0.28, H 14.80±1.47, HS 10.90±1.91, DS 6.87±0.68. Moreover, circulating PF4 (ng/ml) at 1 min was as follows: control 109±8, H 873±134, HS 751±34 and DS 473±15. In another group of H pre-treated rabbits, a bolus injection of PS (10 or 20 mg) caused an immediate disappearance in the circulating plasma PF4, from 900±23 ng/ml (1 min after PF4) to 75±11 ng/ml (1 min after 10 mg PS). However, a subsequent H injection 10 min after PS induced a peak release of PF4 (520±21 ng/ml). In a further group of animals pre-treated with HS, the interaction between PS and PF4 was similar to that observed after H treatment. In the last group, pre-treated with DS, the interaction between PF4 and PS was also similar, however, unexpectedly, when 20 mg of PS were given a subsequent bolus of H did not produce any increase of circulating PF4.
We suggest that PS displaces PF4 from its binding sites on H or HS, thus allowing its uptake by the storage sites in the body, from where it can be harvested again after the subsequent H administration. In the presence of DS again PS is able to displace PF4, however the remaining excess of PS could neutralize the subsequent H injection, thus rendering it unable to induce PF4 release.

703

DIFFERENTIAL NEUTRALIZATION OF UNFRACTIONATED HEPARIN (UH) AND LOW MOLECULAR WEIGHT HEPARINS (LMWHS) BY HUMAN PLATELET FACTOR FOUR (PF-4) A. Racanelli and J. Fareed Departments of Pharmacology and Pathology Loyola University Medical Center, Maywood, Illinois.

Platelet factor 4 neutralization profiles of four newly developed LMWHs, PK 10169 (Pharmuka, Gennevilliers, France) CY 216 (Choay, Paris, France), KABI 2165 (KabiVitrum, Stockholm, Sweden), OP 2123 (Opocrin, Corlo Italy) and an unfractionated porcine mucosal heparin were studied utilizing amidolytic (anti-factor Xa, anti-factor IIa) and clot based (APTT, Heptest) assays. The neutralization studies were carried out in normal human pooled plasma in two experimental protocols. In one set of experiments, PF-4 (10 µg/ml) was added to varying amounts of UH or LMWH (10-2.5 µg/ml). In the second set of experiments, the concentration of heparins was fixed at 5 µg/ml and varying amounts of PF-4(10-2.5 µg/ml) were added. The relative neutralization profiles of these heparins were assay dependant. The anticoagulant effects of UH and LMWHs as measured by APTT were equally neutralizable. Similarly the anti-factor IIa actions of both UH and LMWH were effectively neutralized, whereas the anti-factor Xa activity of the LMWHs was less neutralizable than the UH. Different levels of residual anti-Xa activity of the LMWHs were seen whereas the UH was effectively neutralized. Interestingly, although the anti Xa activity of the UH and Kabi 2165 as measured by amidolytic and clot based methods were similiar and more potent than the other LMWHs, however there were differences between their PF-4 neutralization profile. At a gravimetric ratio of 2:1 , the anti Xa activity of the UH was completely neutralized whereas the Kabi 2165 was least neutralized of all of the LMWHs. The slope of the PF-4 titration curves in the anti-factor Xa assays for all of the LMWHs were similar and varied significantly from the UH. PF-4 neutralization of UH and LMWHs was not dependant on the molecular weight or pharmacopoeial potency (USP or anti-factor Xa). These results show that UH and LMWHs exhibit a dissociation between the anti-factor Xa and anti-factor IIa neutralization which may contribute to their sustained antithrombotic effects.