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TISSUE THROMBOPLASTIN-INDUCED REVERSIBLE DIC AS AN IN VIVO MODEL OF THROMBIN GENERATION AND INHIBITION.

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We have tried a kinetical approach to characterize the dynamics of thrombin generation, thrombin action and heparin-enhanced inhibitors in vivo. Integrals (φ) of free thrombin concentration over time were calculated from fibrinogen decrease to characterize tissue thromboplastin-induced DIC in the dog. DIC with $\varphi <$ 8nMmin was reversible. Dynamic thrombin inhibition (DTI), measured as pseudo-first order rate constant of thrombin inactivation by plasma (baseline DTI = 6.31± 0.79/min, n=30) increased to 68.69±59.98/min (n=7) with heparin (H) and to 22.48±14.91/min (n=5 with pentosan polysulfate (PPS). DTI correlated significantly with heparin doses ($p < 0.002$), and with the prolongation of APTT ($p < 0.02$) and of prothrombin time ($p < 0.05$). The efficiency β of tissue thromboplastin (TP) to trigger DIC (φ per ml TP) was reduced from β =3.81±3.16nMmin/ml to 0.74±0.52nMmin/ml by H ($p < 0.01$) and to 1.16±1.10nMmin/ml (n.s.) by PPS. As expected from the product DTI·φ = 23.4±13.8nM there was no detectable decrease of prothrombin, of the combined activity of antithrombin III + heparin cofactor II (ATHC) or of heparin cofactor II (HC, specific assay by dermatan sulfate activation) in reversible DIC without glycosaminoglycan protection. However, increasing doses of TP at constant PPS protection induced a statistically significant and persistent decrease of prothrombin by 17.6±9.9% and of HC by 20.4±8.8% indicating HC enhancement by PPS in vivo. The model is suitable for the study of glycosaminoglycans and thrombin inhibitors.

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THE MECHANISM OF THE HYPERCOAGULABLE RESPONSE TO HAEMORRHAGE

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Gastrointestinal haemorrhage causes a hypercoagulable state and early blood transfusion increases both rebleeding and transfusion requirements [1]. The role of x- and B-adreno-receptors in the mechanism of this hypercoagulable state and the effects of infusions have been studied in NZW rabbits.

Haemorrhage was simulated by aspirating 20% of the blood volume from the median ear artery. Coagulation was measured before haemorrhage and at 30-minute intervals using the Biobridge Impedance Clotting Time (ICT). Three different groups of rabbits were used: (a) normal controls, (b) B-blocked with propranolol 1mg/kg, and (c) x- and B-blocked with phentolamine 3mg/kg and propranolol 1mg/kg. Equal volumes of (i) Haemaccel, (ii) fresh blood or (iii) stored blood were then given 30 minutes after haemorrhage.

Mean ± sem ICT was 6.0±0.15 minutes before haemorrhage, with no difference between the groups, suggesting that x- and B-blocking drugs have no effect on baseline coagulation. Haemorrhage produced a hypercoagulable state with the mean ICT shortened from 6.1±0.2 to 2.2±0.2 minutes ($p<0.01$). This response was significantly reduced to 5.2±0.4 and 5.1±0.2 minutes by both B-blockade and combined x- and B-blockade respectively.

Haemaccel had no significant effect on the hypercoagulable state, with the ICT going from 2.6±0.2 to 2.8±0.3 minutes. However, fresh and stored blood both reversed hypercoagulability with ICT increasing from 2.6±0.3 to 4.6±0.2 and 5.1±0.1 minutes respectively.

In this model the hypercoagulable response to haemorrhage appears to be mainly B- mediated and is partially reversed by both fresh and stored blood, but not by Haemaccel.

1. Blair SD, Janvrin SB, McCollum CN, Greenhalgh RM (1986). Br J Surg 73: 783-785.

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IN VIVO CHANGES IN SYSTEMIC PCO_2 AND PO_2 INFLUENCE THE THROMBOEMBOLIC REACTION FOLLOWING WALL PUNCTURE IN VENULES BUT NOT IN ARTERIOLES.

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Changes in pH and pCO_2 influence the aggregation of blood platelets in response to various agents in vitro. In the present study intravital video-microscopy was used to investigate whether changes in systemic blood gas values influence the thromboembolic reaction in vivo as induced by vessel wall injury.

The microtrauma was induced by puncturing the walls of microvessels in the rabbit mesentery (diameter range: 20-40 µm) with glass micropipets (tip diameters: 6-8 µm). The thromboembolic reactions were compared in two groups of anesthetized rabbits. The control group was ventilated to keep the blood gas values within normal ranges (means: pH=7.40, pCO_2=32.9 mmHg, pO_2=104.7 mmHg). The experimental group breathed spontaneously (mean blood gas values: pH=7.34, pCO_2=50.5 mmHg, pO_2=48.1 mmHg). The pCO_2 and pO_2 values were significantly different between both groups.

In arterioles and venules of both groups bleeding and thrombus formation started immediately following wall puncture. Bleeding times were short (medians between 1.0 and 2.6 s). Parts of the thrombi started to embolize between 11.4 and 18.2 s following wall puncture (medians). In the control group embolization continued for 101 s in the arterioles and 17 s in the venules; during these periods 6 and 1 emboli were produced, respectively (all median values). In the experimental group the duration of embolization in the arterioles was 143 s in which period 7.5 emboli were produced, values not significantly different from control. In the venules of the experimental group embolization and hence platelet reaction went on uninhibited during the whole observation period of 600 s and 30 emboli were produced. Fluid dynamic factors cannot explain the differences in thromboembolic reaction between the control and experimental venules; vessel diameters and red blood cell velocities were not significantly different between both groups. Therefore, it is likely that the change in thromboembolic reaction in the venules results from the changes in systemic pCO_2 and/or pO_2. The different reactions in arterioles and venules in response to the altered systemic blood gas values might arise from different reactions in the vessel walls.

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EFFECT OF MCI-9038, A SELECTIVE THROMBIN INHIBITOR, ON CEREBRAL MICROCIRCULATION AFTER CEREBRAL ISCHEMIA IN RATS.

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MCI-9038, a synthetic thrombin inhibitor No. 805, has been shown to be effective for various thrombotic diseases including cerebral thrombosis in acute stage. In this report, we studied the effect of MCI-9038 on disorders of cerebral microcirculation in rats generated by the method of Pulsinelli et al. One day after both vertebral arteries were electrocauterized with electrocautery needle through the alar foramina, bilateral carotid arteries were occluded with Vari-angle aneurythm clips to induce hemispheric ischemia, which was confirmed by electroencepharograms becoming isoelectric. Thirty min. after bilateral carotid artery occlusion, clips were removed to restore carotid blood flow and 5 min. later India ink was infused to detect no-perfusion region. The brain was removed and no-perfusion area (NPA) was measured for 10 colonal sections of the brain. While NPA in the control group was 14.6 ± 0.7 % of the total area of 10 colonal sections, MCI-9038 significantly reduced NPA to 5.8 ± 2.1 % at 5 mg/kg i.p. and 6.3 ± 1.3 % at 10 mg/kg i.p. Heparin at 50 and 100 u/kg i.v. and tissue culture urokinase (TCUK) at 48,000 and 96,000 u/kg i.v. did not reduce NPA. Electronmicroscopical observation revealed the platelet aggregates occluding the micro vessels in the region of no-perfusion, suggesting that disorders of cerebral microcirculation in this model resulted from the obstruction of blood flow by micro platelet aggregates. MCI-9038 is considered to improve the cerebral microcirculation by the inhibition of the formation of platelet aggregates. Since MCI-9038 does not inhibit platelet aggregation induced by collagen, ADP or arachidonic acid but by thrombin potently, thrombin is considered to take an important role to form the micro platelet aggregates in this model. The difference in the effectiveness between MCI-9038 and heparin is discussed.