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TITLE: EFFECTS OF PROTAMINE ON VASCULAR SMOOTH MUSCLES OF THE HUMAN MESENTERIC ARTERY

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Systemic hypotension is commonly observed in association with protamine administration after cardiopulmonary bypass. However, little information is available concerning the action of protamine on vascular tissues[1]. We therefore studied the effects of protamine on isolated human mesenteric artery segments both in the absence and in the presence of heparin.

With both the institutional approval and informed consent of each patient, the mesenteric arteries (diameter 0.5-0.7mm) were isolated from five rectal cancer patients (aged 45-74 yr) immediately after colectomy. No patient suffered from hyperlipidemia or any cardiovascular disease. The endothelium-denuded circular strips were prepared as previously reported[2]: its denudation was verified by the disappearance of an ACh-induced relaxant response. Isometric tension was recorded by attaching the strips to a strain gauge in a chamber containing a modified Krebs solution equilibrated with a 95%O_2/5%CO_2 gas mixture (pH7.4). All experiments were performed at 32°C to prevent early deterioration of the tissue. The effects of protamine on 1 μM norepinephrine (EC_{80}, NE)-, 10mM caffeine- and 40mM K^+-induced contractions were studied. The experiments with 40mM K^+ were performed in the presence of 0.1 μM tetrodotoxin and 3 μM guanethidine. To our knowledge, the dissociation constant for the binding of heparin to protamine has not ever been reported[3]. Therefore, in order to minimize the effect of "free" protamine and to examine only the effects of heparin-protamine complex, the experiments with heparin were performed in the presence of the highest possible concentration of heparin (21-700 U/ml: 1.4 U/μg protamine): high concentration (>700 U/ml) of heparin itself inhibited both high-K^+- and NE-induced contractions. The strips were prestretched to an optimal resting tension and after a 60 min equilibration period, the experiments were started. We first studied the effects of protamine on NE- or 40mM K^+-induced contractions in the presence of extracellular Ca^{2+} (2.6mM): figure 1 shows the study protocol, where each stimulant was applied every 8 min for a period of 2 min with the protamine being applied after the amplitude of each contraction became constant. Next, we studied the effects of protamine on NE- or caffeine-induced contractions in Ca^{2+}-free solution containing 2mM EGTA: according to the above results, protamine was applied to the strips in the Krebs solution for a sufficient time in order to exert its maximal effect before the removal of Ca^{2+}, and the NE or caffeine was applied 2-min after the removal of Ca^{2+}.

The protamine-induced inhibition was expressed as percent (%) change from the amplitude of each contraction before the application of protamine. Data was analyzed by an analysis of variance with the Student's t test or the Cochran-Cox test. A level of $p<0.05$ was considered significant.

Protamine time- and concentration- dependently inhibited both the NE- and 40mM K^+-induced contractions in the Krebs solution in the absence of heparin (Fig 2A). In Ca^{2+}-free solution, protamine inhibited NE-induced contractions, but not the caffeine-induced contractions in the absence of heparin (Fig 2B). These inhibitions were almost completely blocked in the presence of heparin (Fig 2).

The results indicate that protamine itself has a direct inhibitory action on the vascular smooth muscles: its inhibitions are probably mediated through the inhibitions of Ca^{2+}-influx and the NE-induced Ca^{2+}-release from the intracellular stores. While, on the other hand, the heparin-protamine complex appears to have no direct inhibitory action on vascular smooth muscles.

Ref: 1) Anesth Analg 64:348-61, 1985 2) Circ Res 61:446-54, 1987
3) Biochim Biophys Acta 883:69-76, 1986

Figure 1

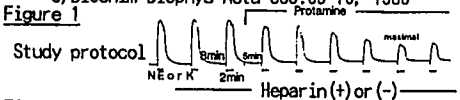
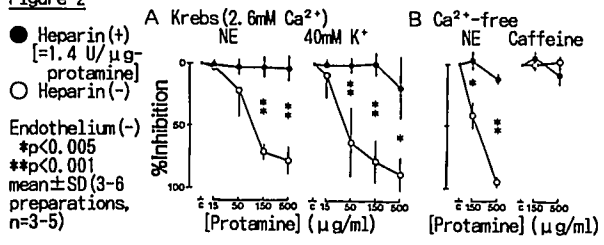


Figure 2



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Title FIBRIN FORMATION DURING CPB WITH AND WITHOUT CIRCULATORY ARREST IN OPERATIONS OF CONGENITAL HEART DISEASES

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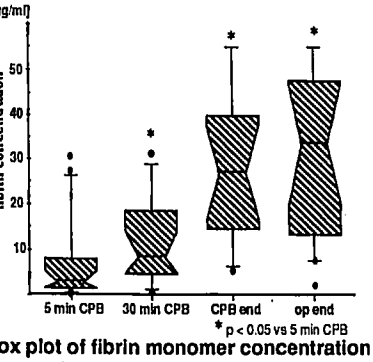
Accurate hemostasis is essential for operations of congenital heart diseases. The contact with the foreign materials of the bypass circuit and the damage to the blood caused by pumps and suction leads to activation of coagulation despite anticoagulation with heparin (1). The influence of cardiopulmonary bypass (CPB) with and without circulatory arrest (CA) on coagulation patterns has not been investigated systematically. The aim of the study was to evaluate the degree of hemostatic activation, especially the degree of fibrin formation during CPB in infants and neonates.

Methods: After institutional approval 20 consecutive patients with a body weight less than 10 kg undergoing open-heart surgery for congenital lesions were assigned to the study. Cardiopulmonary bypass (CPB) was performed in hypothermia (24 °C rectal temperature). 14 Patients were operated on in CA after a rectal temperature of 20 °C had been achieved. The bubble oxygenator was primed with 1 unit of blood. Anticoagulation was achieved with 375 U/kg mucosa heparin under ACT control. Blood samples were taken prior to surgery, 5 and 30 min after onset of CPB (or 15 min after end of CA respectively), at the end of CPB and of operation. To demonstrate the effect of thrombin and plasmin on fibrinogen or fibrin the concentration of fibrin(FbDP)-, fibrinogen(FgDP)- and total(TDP)-degradation products, d-Dimers (DD) and thrombin-antithrombin III complex (TAT) were determined by monoclonal-antibody-based immunoassays. The concentration of fibrin monomer in plasma was detected by a monoclonal antibody directed against the N-terminal α-chain of human fibrin. Nonparametric tests (Friedman test) were used for statistics.

Results: The mean age was 212±189 days (range 10 days to 11/2 year), the body weight 5.4±1.8 kg, the CPB time 84±31 min and the CA time 34±17 min. The ACT 5 min after onset of CPB exceeded 1000 sec in all patients and was 897±441 sec at the end of CPB. All patients showed significant signs of activation of coagulation during CPB (Table). Coagulation patterns did not show significant differences between patients with and without CA. No correlation was found between temperature during CPB and degree of activation of coagulation.



Box plot of fibrin monomer concentration
(norm value: <3μg/ml)

Table 1	TDP [ng/ml]	DD [ng/ml]	FgDP [ng/ml]	FbDP [ng/ml]
preop	730±459	347±201	654±408	195±220.
5 min CPB	1185±1457	348±645	673±453	197±254
30 min CPB	1990±1737*	466±583	1109±710*	899±759*
CPB end	6567±3428*	1422±861*	3051±3278*	4238±3051*

* p< 0.0.5 vs preop

Discussion: Significant fibrin formation during CPB was detected in all patients. Signs of activation of blood coagulation and fibrinolysis were evident despite a clinically sufficient anticoagulation with heparin. Similar but ACT depending results were found by others (2) in adult patients. CA did not influence the degree of activation. Stimulation of hemostasis caused by the damaging effect of CPB may lead to increased bleeding tendency which could jeopardize clinical outcome. Amelioration of anticoagulation by additional drugs or more physiological artificial surfaces seems to be desirable.

References 1. Dietrich, W et al., Anesthesiology 69 (1988) A 120
2. Young, J et al., Ann Thorac Surg 26 (1978) 231