Title : ADVERSE CARDIOPULMONARY EFFECTS AND INCREASED PLASMA THROMBOXANE LEVELS FOLLOWING THE NEUTRALIZATION OF HEPARIN WITH PROTAMINE IN AWAKE SHEEP ARE INFUSION RATE-DEPENDENT

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Introduction. The neutralization of heparin anticoagulation with protamine is occasionally accompanied by major acute hemodynamic and pulmonary effects characterized by pulmonary vaso- and broncho-constriction and systemic vascular collapse (1). We recently developed an animal model producing a consistent and reproducible transient biological and physiological reaction, including complement activation, circulating leukopenia, increased plasma thromboxane B_2 (TxB_2) levels, and pulmonary vasoconstriction following a neutralizing dose of protamine administered by bolus intravenous injection *only five minutes* after intravenous heparinization in sheep (2). The aim of the present study was to evaluate the dependency of the observed characteristic reaction on the rate of infusion of protamine administered intravenously and its relationship to the pharmacokinetic pattern of chemical and biological heparin neutralization in sheep.

Materials and Methods. Six adult sheep (weight 30-40kg) were surgically instrumented for chronic studies with vascular catheters introduced into the thoracic aorta, pulmonary artery, and right and left atrium. A transit-time ultrasonic blood flow probe was placed around the main pulmonary artery for continuous determination of cardiac output. Circulating platelets and leukocytes were measured by phase microscopy. Blood gas tensions and pH were analyzed by an automated AVL 940 Radiometer oximeter. Plasma levels of TxB_2 were determined by standard radioimmunoassay. Plasma heparin levels were determined by a colorimetric assay for chemical heparin based on the metachromasia of the biologic dye azure A (3). Parallelly, the biological, i.e. anticoagulant activity of heparin was detected by measuring the activated clotting time (ACT) of whole blood with a hemochron system. The studies were carried out at least three days after the surgical preparation, with the animals standing in a specially adapted cage for chronic studies. The heparin solution in the vascular catheters was withdrawn and the catheters flushed with normosaline. Bovine lung heparin at a dose of 200IU/kg was injected intravenously over 10sec five minutes before the start of protamine administration (time=0). On separate experimental days, protamine sulfate was given at the same dose of 2mg/kg, but infused over four different time periods: 3, 30, 300sec, and 30min. On an additional session, protamine was administered over 3sec without prior heparinization, in order to assess the effect of protamine alone. The sequence of the sessions was randomized and performed blindly. Statistical comparison over time and between treatment groups was conducted by a one-way ANOVA, with significant ($P<0.05$) differences detected by Duncan's multiple comparison test.

Results. The main results of this study are illustrated on Fig. 1. Injecting protamine in unheparinized sheep produced no change in any of the measured variables. In contrast, when protamine was injected over 3sec five min after heparin, it induced a transient pulmonary hypertension, increased pulmonary and systemic vascular resistance, decreased cardiac output without change in left atrial pressure, increased plasma TxB_2 levels, leukopenia, and hypoxemia. All other measured variables were not significantly affected by protamine injection. Administering the same amount of protamine after heparin at a lower infusion rate significantly attenuated and delayed all components of the adverse response to protamine, and this in a dose-dependent fashion (Fig. 1). When protamine was infused over 30minutes, no significant changes in any of the measured variables were noted. The time course of plasma heparin levels indicates that chemical heparin was completely neutralized within the time period of protamine infusion. Similarly, ACT values increased >600sec in the four heparinized groups, and decreased to 104±4 (3sec group), 129±12 (30sec), 114±11 (300sec), and 412±87 (30min, $P<0.05$ from other groups) at 10 min after the start of protamine infusion.

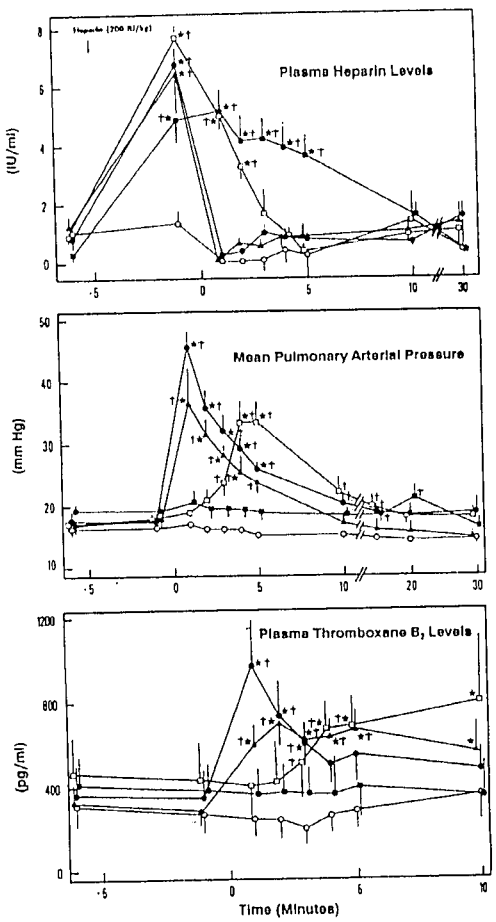

FIGURE 1. Heparin neutralization with protamine infused over 3sec (●), 30sec (▲), 300sec (□), and 30min (■). Heparin is injected at -5min, protamine infusion is started at 0 min. Data points represent mean ± SE values; n=6 in each group. *$P<0.05$ from baseline (time=-1); †$P<0.05$ from unheparinized group (○).

Discussion. These results demonstrate that the *rate* of generation of heparin-protamine complexes (as detected by changes of plasma levels of chemically active heparin) during i.v. protamine infusion is a determinant factor to generate sufficient mediators to initiate a characteristic physiologic response in sheep, including systemic and pulmonary vasoconstriction, TxB_2 generation, and leukopenia. Infusing a neutralizing dose of protamine over 30 min allows to avoid these adverse reactions.

References. 1. Lowenstein E, *et al.* Anesthesiology 59:470-3, 1983
2. Morel DR, *et al.* Circ Res 62:vol 5, 1988 (in press)
3. Klein MD, *et al.* Analytical Biochem 124:59-64, 1982

Supported by the Swiss National Science Fund no. 3.812-0.86.