TITLE: PTT-PROTAMINE DOSE RESPONSE CURVE WITH AND WITHOUT HEPARIN IN VITRO

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Introduction: It has been known for a long time that protamine sulfate has an anticoagulant action in the absence of heparin. Although the anticoagulant action of protamine may not be important clinically, we were interested in how protamine affects activated partial thromboplastin time (APTT) in vitro in the presence and absence of heparin.

Method: Part 1 Study with Heparin: Venous blood was obtained from 19 mongrel dogs. Each sample was anticoagulated with sodium citrate and plasma was separated by centrifugation. Seventeen plastic tubes were prepared for each dog's plasma. 1.5 ml of the plasma was introduced into each plastic tube and 20 μg (=2 units: considering 1000 units as equivalent to 10 mg of heparin for convenience) of sodium heparin (Upjohn Co.) was mixed. The protamine sulfate (Upjohn Co.) was then added to this mixture, increasing its dose from zero to 200 μg in 1.5 ml plasma. APTT for each mixture was measured using a B.B.L. Fibrometer and General Diagnostic automated APTT reagent. Part 2 Study Without Heparin: Plasma was obtained from 13 mongrel dogs. APTT was measured for plasma with increasing doses of protamine ranging from zero to 180 μg in 1.5 ml plasma.

Results: Three phases were observed in the APTT-protamine dose response curve when heparin was present (Fig. 1). Phase 1: protamine neutralized the anticoagulant effect of heparin. Phase 2: APTT values leveled after the maximal antiheparin effect of protamine was obtained. There were no statistical differences in APTT values at 20, 40 and 60 μg of protamine in 1.5 ml plasma. Phase 3: protamine exerted its own anticoagulant action. APTT increased from 36.5±3.2 sec (mean ± SE) to 41.0±4.2 sec when protamine dose was increased from 180 μg to 200 μg. When heparin was absent, protamine began to exert its anticoagulant effect after its dose reached 20 μg in 1.5 ml plasma (Fig. 2). APTT increased from 54.5±3.3 sec to 62.0±3.0 sec when protamine dose was increased from 160 μg to 180 μg.

Discussion: Protamine is a polycationic basic protein which combines with heparin by non-selective electrostatic complexation forming a stable compound.[1] The number of milligrams of protamine required to neutralize 1 mg of heparin depends on many factors including different heparin and protamine preparations, in the in vivo study versus the in vitro study. In our in vitro study, protamine acts as an antiheparin until milligram concentration of protamine becomes equivalent to that of heparin. This agrees with the statement in the protamine vial insert. During Phase 2 in Fig. 1, APTT remained unchanged until protamine concentration reached approximately 3 times that of heparin. This might indicate that protamine molecules are still binding to the already formed heparin-protamine compound without increasing the free protamine molecules. It might also indicate that protamine has wide safety ranges clinically to neutralize the anticoagulant effect of heparin where APTT is concerned. The increase in APTT by the same increment of protamine concentration is less when heparin is present. It might be possible that the heparin-protamine compound may inhibit the anticoagulant effect of protamine itself. In summary, (1) When heparin exists the anticoagulant effect of protamine does not appear until the concentration of protamine exceeds approximately 3 times that of heparin in vitro. (2). The anticoagulant effect of protamine is more potent in the absence of heparin.

Fig. 1

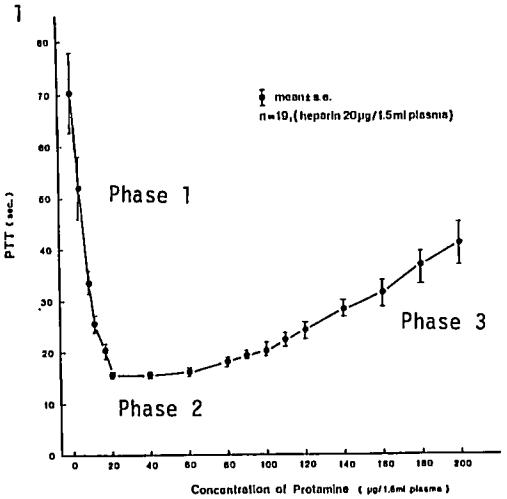
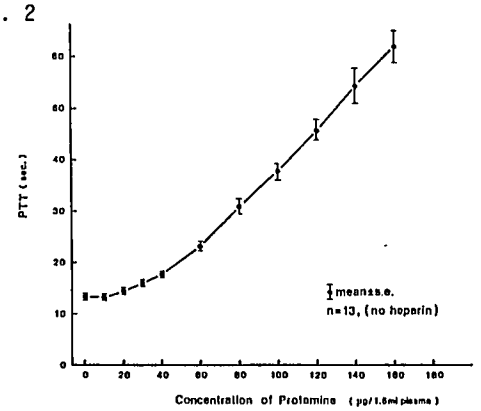


Fig. 2



Reference:

1. Poon MC, Hurst RE, Rives MS: Platelet factor four and protamine sulfate neutralization of heparin fractionated according to anionic charge density. Thromb Haemostas 47:162-165, 1982