Freezing of Plasma: Proposal Regarding Effect on FVIII Yield in Cryoprecipitate

Introduction: In their recent review of FVIII developments (1), Foster and McIntosh indentified as a high priority for the future FVIII developments, need to improve the initial recovery of FVIII activity from plasma.

AIM

During studies for the development of heat treated factor VIII, McIntosh et al discovered that the methods of freezing of factor VIII solutions was critical to the recovery of factor VIII (2). It was predicted that a similar effect of freezing mode might be seen for the effect of plasma freezing on yield of factor VIII in cryoprecipitate (2). Preliminary small scale (20ml) studies (3) demonstrated that supercooled plasma (with a slow overall freezing rate) maintained as high a yield of FVIII as more rapidly frozen plasma but failed to show any benefit of supercooling. This was confounded by variable cryoprecipitate yield for the -40° shelf frozen versus supercooled plasma samples assessed using water bath thawing. A higher yielding cryo preparation method (eg thaw siphon, which models the bulk thawing method used at PFC) should reduce variability.

The aim of the proposed study is to assess the effect of standard vs against supercooling modes of plasma freezing on factor VIII yield in thaw-siphon cryoprecipitate.

PROPOSAL

First determine conditions for supercooling of standard FFP donations. Paired samples of standard FFP (≥200mLs, n≥10) to be frozen by standard method (direct placement at one freezing temperature to ensure freezing to below -30° within 30 minutes) or with supercooling (preliminary cooling to -5 to -10° until equilibrated, then very rapid freezing to -40° ie two stage freezing eg in programmed blast freezer). Subsequently prepare cryoprecipitate by thaw siphon method (Vox Sang 37 235-243, 1979). Assess plasma, supernatant and cryo for factor VIII content, total protein and volume.

Experiments on the effects of adding small amounts of precipitants, such as PEG or PVP, to plasma prior to freezing to increase the cryo precipitating effect of freeze concentration (± supercooling) could demonstrate an independent effect.