

A novel recombinant factor VIII produced by a human cell line functionally restores coagulation deficits in a mouse model of haemophilia A.

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A novel cell line derived from the human embryonic kidney cell line HEK293 has been generated which secretes human B-domain-deleted clotting factor VIII (Octagene SF). To quantify the functional activity of this factor, we have established an in-vivo assay using a mouse model of haemophilia A. Mice bearing a targeted deletion in exon 16 of the FVIII gene received intravenous injections of purified Octagene SF or ReFacto® at different doses (50, 300 and 600 IU/kg). After different time intervals ranging from 3 to 70 hours, blood samples were withdrawn either retro-orbitally or intracardially. Changes in coagulation efficiency were quantified measuring clotting time with a one-stage aPTT assay. Octagene SF significantly reduced the clotting time even at the lowest doses and after various time intervals. A dose-response curve could be established where higher Octagene SF doses correlate with shorter clotting times. In order to test the immunological response elicited by injection of recombinant FVIII, mutant mice have been subjected to a Bethesda assay. Following intravenous injection, blood samples were weekly withdrawn and the immune response quantified via ELISA. Our results show that Octagene SF displays stability and clearance kinetics comparable to those of ReFacto®, indicating that the protein is physiologically active in vivo and providing strong support for its therapeutic potential in humans.