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Analysis of blood coagulation in the zebrafish.

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Abstract

The zebrafish (Danio rerio) is a unique animal model in which saturation mutagenesis has been used to identify genes involved in vertebrate development. The relevance of the zebrafish as a genetic model for hemostasis depends, in large part, on the degree of similarity between the zebrafish and mammalian systems. The diminutive size of the zebrafish poses technical problems for analysis of coagulation. This study describes methods to obtain citrated whole blood and plasma from the zebrafish, analyze in vitro coagulation in small plasma volumes, obtain uniform dosing of zebrafish with oral anticoagulants, and demonstrate specific factor activities via chromogenic assays. Analysis of the zebrafish system demonstrates the presence of both the intrinsic and extrinsic pathways of coagulation, evidence for prothrombin, factor X, protein C, antithrombin, and heparin cofactor II activity, and a requirement for vitamin K dependent gamma-carboxylation of zebrafish hemostatic proteins. Induction of a morphologically recognizable bleeding phenotype by warfarin treatment is also demonstrated. Characterization of zebrafish coagulation provides evidence that major hemostatic pathways are conserved between zebrafish and man. These similarities indicate that the zebrafish is a relevant genetic model for identification of novel genes involved in hemostasis and thrombosis.

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