

Animal Models of Bleeding and Tissue Repair

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Animal models have allowed us to make great progress in hemophilia research and care, Dr. Maureane Hoffman said. They allow us to study a phenomenon that is relevant to patients, without actually conducting experiments on those patients, she added.

A number of animal models have been used to study the local complications of excessive bleeding such as hematomas (severe bruises), joint damage and impaired wound healing. There are three basic types of models: (1) the animal has a naturally-occurring condition that is similar to bleeding disorders in human, (2) the condition is produced in the animal by pharmacologic treatment, and (3) the condition is produced in the animal by genetic manipulation (knockout mice).

Dogs were among the first species that were used as animal models in hemophilia research, because they have some of the same inherited bleeding disorders that humans do – hemophilia A and B, von Willebrand disease, and other factor deficiencies, Dr. Hoffman said. The Chapel Hill colony, which was established in 1948, was used to make the first observations about the biology and natural history of hemophilia. For example, this model allowed us to observe that hemophilia has the same fundamental biology in females as in males. This is rare in humans, but easier to study in colonies. Dogs also experience arthropathy the way humans do. The dog model is also useful to study different types of therapy; treatments from early preparations of factor VIII concentrates all the way to gene therapy trials have been evaluated in this model.

Dogs will continue to be used for dosing studies and for developing diagnostic tests, Dr. Hoffman said. However, while it may be easier to compare what happens in dogs to humans, it can be difficult to reproducibly measure their bleeding tendency. They are also very expensive and labour-intensive to maintain when you consider how many animals are needed to conduct proper statistical work, so research on hemophilia has been conducted in other small animal models. Mice, rats, and rabbits can be injected with an antibody against any given coagulation factor and thus used as a model. More recently, knockout mice have been developed for each of the common coagulation factor deficiencies, which are cheaper and easier to maintain than larger animals. Mouse models don't translate as well to humans, though, i.e. they don't experience the same symptoms (such as spontaneous joint and tissue bleeds) as do people with hemophilia. Bleeding patterns are also not always easy to reproduce and measure, which can make it difficult to get statistically significant data. The recently published vein transection model of bleeding and rebleeding (Sheehan & Whinna, *Blood*, 2008) has promise in this regard, Dr. Hoffman said.

Bleeding and tissue repair

Theoretically, there are good reasons to expect people with hemophilia (who have impaired clot formation) to have impaired wound healing as well. The fibrin clot provides the framework on which tissue repair occurs. Then fibrin degradation products call in inflammatory cells. Some activated factors have other activities besides making blood clot. For example, thrombin has cytokine and growth factor like activities. Each stage in the host response prepares and influences the next.

However, when Dr. Hoffman asked hematologists, “Do people with hemophilia have normal wound healing?” she found that the more experienced clinicians said no, it is impaired. Younger clinicians who have not seen untreated or inadequately treated patients, on the other hand, said it was normal.

Only a limited number of studies have examined the relationship between bleeding and tissue repair directly, Dr. Hoffman said, so her group developed an experiment with FIX knockout mice. They found that hemophilic mice often developed subcutaneous hemorrhage, that there was a delay in the influx of macrophages to the bleeding site, and that wound healing was, in fact, delayed. These results are reproducible, she noted. Her team was also surprised to find an increase in angiogenesis, i.e. the formation of new blood vessels.

In other experiments, Dr. Hoffman and her group then studied the effect of fibrin formation on wound healing. Since the production of fibrin is reduced in hemophilia B (HB) mice, they hypothesized that restoring thrombin generation during initial hemostasis would normalize fibrin production and subsequent wound healing. However, when they gave HB mice a dose of either FIX or VIIa before punch biopsy, they found that this did not make wound size return to normal. They also found that the amount of iron in the wounds of HB mice was higher, and it stayed high longer than in normal mice. Even after the wound was completely healed, iron remained. Iron is linked to angiogenesis and the development of arthropathy, leading to an increased risk of recurrent bleeds, Dr. Hoffman said. They concluded that normal wound healing requires normal hemostatic function for an extended period of time, and not only at the time of initial clot formation.

Late bleeds after healing

Ongoing hemorrhage or late (re)bleeding, which occurs even after a wound is healed in HB mice, may be linked to angiogenesis, Dr. Hoffman said. New blood vessels are very delicate and leaky, and they disrupt the structure of existing blood vessels as they sprout. Her group also discovered that tissue factor (TF), which evidence suggested would increase during angiogenesis, actually disappears from the surrounding tissue. This may also contribute to the increased risk of repeated bleeding in hemophilia, she said.

“Could both late re(bleeding) in HB mice and recurring joint hemorrhage in humans be due to a cycle of inflammation, iron accumulation and angiogenesis?” Dr. Hoffman speculated. The most effective way to break the cycle, she said, would be to prevent the initial hemorrhage in the first place. Whereas extended factor therapy may prevent rebleeding related to angiogenesis, only up-front prophylaxis can prevent the initial bleeding episode, she concluded.