Viral gene therapy for haemophilia

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Researchers from the University of North Carolina (Chapel Hill, NC, USA) have used a genetically engineered virus to infect mouse cells and produce high quantities of a clotting protein similar to that lacking in people with haemophilia. If human gene therapy studies using this technique prove successful, haemophiliac patients might no longer need daily injections of the proteins they lack.

Haemophilia is an X-linked recessive genetic disorder that affects 1 in 5000 male births and results in a disorder of clotting factors. The condition is characterized by patients bleeding spontaneously into joints and muscles, and on rare occasions their brains, which can prove fatal.

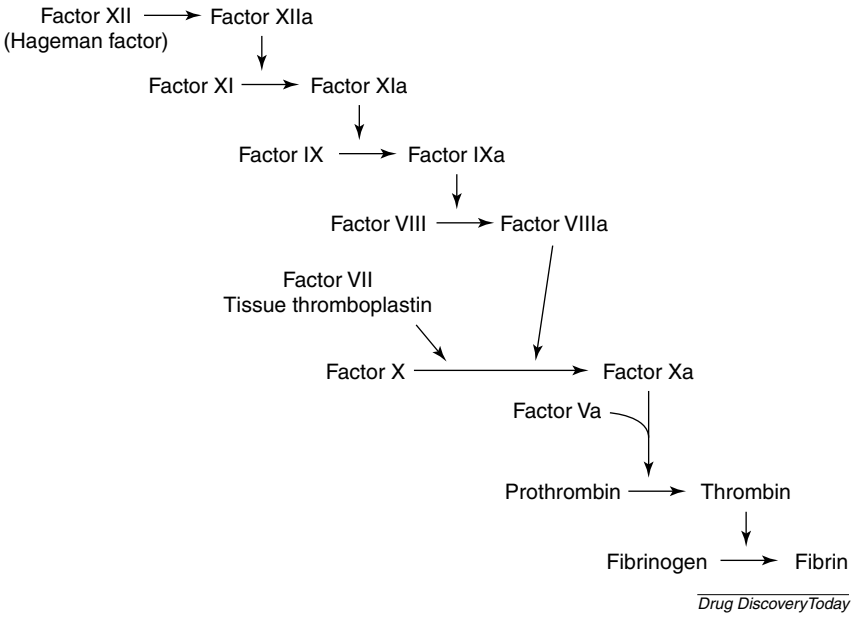

Figure 1. The coagulation cascade, which leads to the conversion of fibrinogen to insoluble fibrin.

Pathophysiology of haemophilia

Haemophilia A (affecting 80% of sufferers) is caused by a deficiency of the blood protein factor VIII, while the rarer haemophilia B (affecting 20% of sufferers) is caused by a deficiency of factor IX. A fundamental reaction in the clotting of blood involves the conversion of the soluble plasma protein fibrinogen to insoluble fibrin which, via an intrinsic pathway or cascade, requires the activation of various factors including factors VIII and IX (see Fig. 1).

'Lacking either factor VIII or factor IX has an identical result. The severity of the haemophilia matches the quantity of protein produced and reflects the different mutations that exist: if the protein is absent or greatly truncated, you get the severe phenotype,' explains Christopher E. Walsh, senior author of the study, Assistant Professor of Medicine at the UNC-CH School of Medicine and Clinical Director of the UNC Gene Therapy Center.

Current treatments for haemophilia involve injecting patients with the missing proteins obtained from either fractionated blood or from recombinant DNA technology. In the 1980s, haemophiliacs commonly became infected with hepatitis B and C and HIV from these treatments. Although infectious agents can now be removed using solvents or detergents, procedures are not always effective and newly evolved viruses could be transmitted. 'Even with recombinant DNA technology, human serum albumen is commonly derived from plasma and used to stabilize factor VIII, creating potential problems of infection,' says Walsh.

Using viral gene therapy

Walsh and his team believe that the use of gene therapy to deliver the genes that make the missing proteins would overcome many of the concerns surrounding infection. They have been using the recombinant adeno-associated virus (rAAV) to carry human and canine factor IX into mouse cells. AAV infects both dividing and non-dividing cells and persists in tissues such as skeletal muscle, retina, liver, brain and vasculature[1]. The team is initially working with factor IX. 'The gene for factor IX is much smaller – 1000 base pairs (bp), compared with 7000–9000 bp for factor VIII – making it much easier to manipulate,' says Walsh.

Of the six AAV serotypes, serotype 2 (AAV2) is best characterized and has been the most commonly used in gene transfer studies. The team has previously demonstrated that AAV2 targeting skeletal muscles produces functional factor IX in large animal models, but at less than therapeutic levels[2]. They are now working to see if alterations in the AAV capsid would affect skeletal muscle transduction and factor IX secretions[3].

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Using an identical canine factor IX expression cassette, they cross-packaged the genome into virions generated from five AAV serotypes and injected them into the muscles of immunodeficient mice and FIX knockout mice. Surprisingly, while the time-to-onset of detectable serum levels appeared the same for all serotypes, types 1, 3 and 5 produced 100–1000-fold more canine factor IX than type 2. 'Simplistically, we think that this could be because more muscle cells are capable of taking up the virus in types 1, 3 and 5,' says Walsh. This would directly result in increased protein leaching into the bloodstream. Furthermore, using green-fluorescent-protein genes in the expression cassette, they found that AAV type 2 could only attach to the slow-twitch muscle fibres, whereas types 1, 3 and 5 also attached to fast-twitch fibres. The team is now repeating the experiments in haemophiliac dogs and primates to ensure there are no key differences in muscle composition between mice and other animals.

Future studies

The results of these studies will be used as a benchmark to determine whether a clinical trial is warranted. Studies will be needed to show that the gene persists in making proteins at high levels (so far, results in mice look promising after six months) and that there are no obvious side effects of the introduced virus or muscle damage when tested in larger animals.

'The mice in the experiment were immunosuppressed to avoid the production of antibodies against the virus or protein. If we move into clinical trials, this will be a major hurdle to overcome,' says Walsh. If ultimately successful, Walsh believes the delivery system could be used for other diseases that require an increase in circulating levels of a protein. For example, in cancer, he suggests the method could be used to produce anti-angiogenesis proteins.

References

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Flower power: fact or fiction?

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Key researchers from both sides of the Atlantic have called for the urgent revision of the regulations of herbal medicines. These remarks were made during a recent seminar in London (UK), sponsored by the Royal Society of Chemistry (London, UK) and the American Chemical Society (Washington, DC, USA).

The dietary supplements market has been one of the fastest growing areas in the healthcare arena in recent years, particularly in the USA where 50% of the population regularly take supplements ranging from multivitamins for general wellbeing to alternative herbal remedies for chronic and serious disease. However, for the majority of these products there is little, if any, scientific evidence of efficacy. This, together with unregulated advertising and media coverage, is a potential risk to the public who might be led to believe that herbal remedies are as effective and safe as conventional medicine. Furthermore, the lack of a regulatory system for these supplements means that the quality and purity of the extract, its toxicity and side effects (e.g. drug–drug interactions, risk to pregnancy) and potential for overdose present a significant risk to the public.

The need for evidence

Edzard Ernst (Chair in Complementary Medicine, Exeter University, UK) presented data from clinical trials that have demonstrated the efficacy of several herbal extracts, such as ginkgo biloba, which has been shown to be beneficial in delaying the chemical deterioration of dementia[1]. Ernst also highlighted the potential problem of drug–drug interactions in patients taking herbal

supplements. For example, it has been reported that St John's wort causes induction of hepatic P450 enzymes, which might reduce plasma levels of concomitantly taken medication[2]. Such interactions are of particular concern because patients often do not inform their doctor that they are taking supplements.

Ernst also discussed how negative data obtained by herbal extract manufacturers might be withheld, leading to

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