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Flushing Out Antibodies to Make AAV Gene Therapy Available to More Patients

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Six men with hemophilia recently underwent intravenous infusion of a vector based on adeno-associated virus (AAV) serotype 8 that expressed factor IX (FIX), which is deficient in such patients. Gene transfer led to levels of expression of FIX that were between 1% and 6% of that in normal individuals for more than two years, and to conversion from severe hemophilia B to mild or moderate disease.[1,2] However, these patients had been carefully selected to exclude those with preexisting natural immunity against AAV in the form of neutralizing antibodies (NAbs). Such antibodies markedly impair gene expression in macaques and redirect vector DNA to the spleen.[3] Of tested human serum samples, 75% have NAb titers of less than 1:10, a level that predicts successful gene transfer following systemic gene delivery.[4] Unfortunately, one in four potential hemophilia gene therapy candidates will be excluded from treatment on the basis of excessive titers of NAb. It is this cohort of patients that Mimuro and colleagues set out to help in a study reported in this issue.[5] They show that accessing the portal vein and purging the liver of venous blood with saline so as to minimize contact of the gene transfer vectors with NAbs can enhance the efficiency of vector transfer.

The liver is integral to most metabolic pathways and the production of many serum proteins.[6] Defects in several of these pathways and proteins lead to human suffering in diseases such as the clotting disorders hemophilia A and B (patients deficient in factors VIII and IX, respectively). The liver is therefore a prime candidate organ for hemophilia gene therapy. Conversely, hemophilia as a disease candidate has attributes favoring therapeutic liver-directed gene transfer. The clinical phenotype is due to a single defective gene product; tight regulation of gene expression is not critical, and levels of gene product above 5% of normal may prevent episodes of spontaneous bleeding.[7] Key translational decisions are the choice of vector and the route of its delivery. Vectors based on AAV still appear to hold the greatest promise for liver gene transfer, with serotype 8 a leading candidate.[8]

To selectively target the liver by an intravascular route, one can access the portal vein or the hepatic artery (**Figure 1**). Approximately two-thirds of hepatic blood flow is derived from the portal vein and one-third from the hepatic artery. The common hepatic artery—or a more selective branch—can be accessed from the extremities via the femoral or brachial arteries; however, intrahepatic flow is regulated by vasoconstriction of the artery and not the portal vein, such that the latter may represent a better choice for perfusion.[9] The portal circulation presents a unique anatomical challenge with regard to intrahepatic vector delivery; one cannot access the portal circulation via peripheral veins such as the brachiocephalic or saphenous vein. Venous blood from the stomach, gall bladder, small intestines, colon, pancreas, and spleen returns via the liver by passing sequentially through venous tributaries (e.g., the superior mesenteric vein or splenic vein) to the portal vein, the liver acini, the hepatic veins, the inferior vena cava, and ultimately the right atrium of the heart before systemic dissemination (**Figure 1**).[10] Humans have an additional consideration regarding direct portal venous vector delivery: collateral venous connections. To completely eliminate venous blood entering the liver, which may potentially prevent expression of vector by NAbs, several collateral pathways should ideally be ligated and divided, including the right adrenal vein, small direct retroperitoneal tributaries, and the phrenic veins.[11]

Mimuro and colleagues[5] chose the portal venous route to test the efficacy of saline flushing in order to avoid the inhibitory effect of antibodies against AAV vectors. They utilized an AAV8 vector with a hepatocyte-specific promoter to express a mutant macaque FIX that could be distinguished from wild-type macaque FIX by a specific antibody. Four cohorts were studied. The first was of macaques without detectable NAbs. Infusion of 5×10^{12} vector genome copies per kilogram, performed via the saphenous vein, resulted in therapeutic levels of expression. Saphenous vein infusion is not a selective liver-targeting method because the blood from the saphenous vein returns to the heart via the inferior vena cava and does not directly enter the portal circulation. In the second cohort, the same vector at a fivefold lower dose was infused via mesenteric venous branches of macaques with low NAbs and resulted in subtherapeutic expression. The mesenteric vein is one of many tributaries leading into the portal vein in macaques and other nonhuman primates. The authors hypothesized that NAbs in the blood neutralized the AAV8 vector in this cohort.

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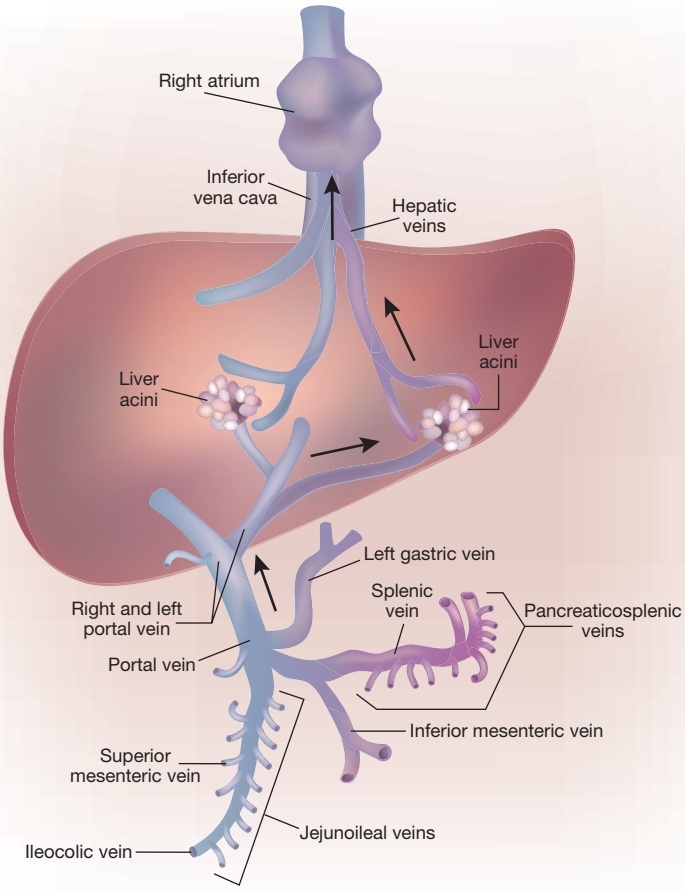

Figure 1 Anatomy of the portal venous system. Omitted is the anatomy of the hepatic arteries and bile ducts. The arrows show the direction of blood flow. In the liver acini (of which there are 90,000 in the adult human), portal blood "percolates" from centrally located terminal portal venules to peripherally located hepatic venules. Data from ref. 9.

To minimize the exposure of the vector to NAbs in blood, Mimuro *et al.* studied two additional cohorts of macaques with low NAbs, utilizing portal vein–directed vector-delivery strategies for flushing the liver with saline to remove blood. One strategy employed direct injection of the AAV8 vector after clamping of the left portal vein; the other involved balloon occlusion of portal venous inflow and flushing of the left hepatic lobe with saline prior to vector injection to remove blood. Both techniques reduced the inhibitory effects of NAbs targeting AAV8. However, only macaques with low NAb titers were studied, and the generalization of the findings to macaques with higher titers of NAbs remains to be evaluated, although very few humans have high NAbs to AAV8.

The three portal-vein strategies utilized by Mimuro *et al.* in macaques are technically feasible in humans but would require general anesthesia and a laparotomy (a large incision through the abdominal wall). Laparoscopic surgery (insertion of cameras and other instruments into the peritoneal cavity via small holes in the abdomen) would be less invasive than laparotomy yet still allow direct access to the portal vein or one of the many tributaries leading into the portal, such as the ileal tributary used by the authors. However, like a laparotomy, laparoscopic surgery requires general anesthesia, and direct infusion into the portal vein is technically difficult and risks injury to adjacent structures, including the biliary and arterial systems. Natural-orifice transluminal endoscopic surgery (NOTES) allows access to the abdominal area without a surgical incision.[12] Instead, an endoscope is passed through a natural orifice (mouth, vagina, anus, urethra). An internal incision in the stomach, vagina, colon, or bladder allows access to the abdominal viscera for diagnostic and therapeutic procedures. NOTES has theoretical advantages over laparoscopy, including faster recovery, shorter hospital stays, lower anesthesia requirements, and avoidance of surface scars and hernias. However, questions about the cost, safety, and advantages of NOTES over established minimally invasive surgical options make it a less likely choice for portal-vein delivery.

Interventional radiological techniques to access the portal vein are well established. In the transjugular approach, a catheter is introduced through the jugular vein and advanced through the hepatic veins through the hepatic parenchyma and into a branch of the portal vein. A balloon catheter can be placed and inflated for temporary proximal occlusion of the vein, such that saline flushing and vector infusion can be performed. Perhaps the most promising method for translating the portal venous flush technique into humans is the percutaneous transhepatic approach, in which direct puncture of the liver is used to advance a needle through the liver parenchyma into a branch of the portal vein. This technique is commonly performed by interventional radiologists for assessment of portal venous pressure, liver biopsy, balloon dilation of portal-vein stenosis, or treatment of portal-vein thrombosis. A balloon catheter could be selectively placed in a branch of the portal vein, inflated for temporary occlusion of portal venous inflow, and flushed with saline before vector delivery.

Looking further ahead, additional techniques of separating vector from NAbs should be considered. During plasmapheresis, for example, each sequential apheresis cycle has been shown to effect a twofold reduction in NAb titers.[13] Such a reduction suggests that one or two cycles would, respectively, make 3% and 9% more patients eligible for AAV transduction.[4] The efficiency of transduction and persistence of AAV vector transduction in liver suggest that a one-time vector delivery—even if more strenuous than cannulation and balloon catheter delivery—might be warranted for individuals with the greatest opportunity to benefit from genetic correction via liver gene transfer.

Drawing on the experience with regional chemotherapy for hepatic cancers not amenable or responsive to other cancer treatment modalities, a more aggressive but potentially more efficient approach would be isolated hepatic perfusion.[14] This technique, using a combination of melphalan and tumor necrosis factor in patients with advanced malignancy, has had an acceptable mortality rate of 4% (ref. 15). The preferred surgical technique skeletonizes the portal vein and common hepatic artery, establishing venovenous bypass through an external circuit similar to that developed for liver transplantation, and perfusion through the hepatic artery.[15] More recently, percutaneous techniques have been developed that have the potential to decrease complexity, cost, morbidity, and convalescence.[16] In addition, the efficacy of regional perfusion as proof of concept has been shown in gene transfer studies.[17]

In summary, translational strategies such as that described by Mimuro and colleagues to minimize the exposure of AAV8 vector to neutralizing antibodies in blood has relevance to humans but will probably require a modified technique, preferably avoiding general anesthesia and laparotomy. In clinical medicine, established interventional radiological techniques offer advantages. A fluoroscopically guided transhepatic approach may provide a better method for implementing this strategy for human subjects with natural AAV8 immunity who are otherwise excluded from treatment. Ultimately, other, more definitive methods of protecting vector from blood may prove to be safe and have superior effect.

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