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Liver Failure

Research is a dynamic activity that creates new ideas. It provides a forum for generating observations and testing why they occur. Because people and their diseases are so diverse, clinical trials are the ONLY WAY it is possible to test whether new ideas about how to diagnose or treat human disease will work. But the process of taking research from bench to bedside is a lengthy one and demands not only vision but also years of team-work and dedication on the part of scientists, physicians and patients
This document presents some of the current research ideas about how stem cells could be used to achieve more cures for liver failure. The content presented is by no means exhaustive and readers may wish to peruse additional web resources or speak with their physicians for more information.

About liver failure

The liver is the largest solid organ and the largest blood reservoir in the human body. One quarter of all the blood leaving the heart is directed to the liver and as blood circulates through the organ, it performs a number of functions that are critical for sustaining life. The liver metabolizes nutrients transported from the intestines, removes waste products from our body, filters toxic substances and drugs from our blood, and helps to maintain the levels of blood sugar, fat and hormones. The liver also participates in the immune response against infection.

The majority of the liver (80 per cent) is made up of liver cells called hepatocytes. These have an average lifespan of 150 days, which means that the liver is constantly renewing itself under normal conditions. Hepatocytes are the powerhouses in the liver, performing most of the critical metabolic functions. These cells secrete glucose and proteins into the blood and bile into the small channels, called canaliculi, that run between the cells. Bile is stored in the gall bladder and is necessary for the digestion and absorption of fats. Other important cell types include the bile duct cells, endothelial cells, stellate cells, pit cells, and macrophages (called Kupffer cells in the liver) that remove debris, pathogens, and damaged blood vessels.

The liver is a very hardy organ that can withstand a great deal of abuse and continue to function. That is why when the liver reaches a state of 'failure', the damage to the structure and function of the organ is irreversible and its effectiveness in maintaining so many of the body's essential functions is diminished beyond repair. Individuals who develop liver failure can die within months of diagnosis.

Liver failure is increasing dramatically and some estimate it will triple in the next 20 years. The Centre for Disease Control and Prevention estimates that 30 million people in the USA suffer from a liver disorder and 27,000 per year die from liver disease. Currently, the most common causes of chronic liver failure are viral hepatitis and alcohol. In Canada, liver disease is the fourth leading cause of death. The most common cause is a condition known as fatty liver disease but Hepatitis B and C are also major causes of chronic liver disease. It is estimated that more than two million Canadians - regardless of age, sex, ethnic origin or lifestyle - will be affected by a liver or biliary tract disease in their lifetime, and upwards of 400 liver transplant operations are performed every year.

Symptoms and Causes

Acute versus chronic liver disease

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There are over 100 different kinds of liver disease. The causes can include alcohol, viruses, obesity, genetics, autoimmune diseases, drugs, toxins and cancer. Liver failure can be the result of an acute condition that develops in a matter of days, or a chronic condition that evolves slowly over time. The causes are different in children as compared with adults. In children, the leading causes of acute liver failure include acetominophen toxicity, metabolic disorders, and autoimmune disease. Chronic liver failure in children is most often caused by a blockage in the tubes that connect the gall bladder to the liver (biliary atresia). In contrast, acute liver failure in adults is most often attributed to viral hepatitis whereas the main causes of chronic liver failure is cirrhosis, caused by alcohol or hepatitis C. A lifestyle of "excess", characterized by obesity and high triglycerides stresses the liver, and contributes to a condition known as fatty liver which is now emerging as a common cause of liver failure in adults.

Alcohol

Worldwide, cirrhosis of the liver caused by alcohol is the leading cause of chronic liver disease. Over a period of time alcohol abuse can induce permanent changes in the liver, which may be characterized by scar tissue or fibrosis and inflammation in a significant portion of the liver. Although this damage is permanent, the liver is a large organ and some portions may still remain unaffected. If the disease progresses, more and more of the liver becomes scarred, the capacity for regeneration in the healthy portion diminishes, and liver failure results. The rise in liver disease and cirrhosis in the younger demographic is particularly alarming and is thought to be associated with binge drinking.

Hepatitis

Hepatitis is inflammation of the liver most commonly caused by viral infection. Hepatitis A virus is found in contaminated food or water. Although the liver usually recovers, hepatitis A virus infection can be fatal. Hepatitis B is a virus transmitted through blood and bodily fluids from mother to baby, by sexual contact and drug use. Today the incidence of new cases of Hepatitis B virus disease is falling due to successful vaccination programs worldwide. Hepatitis C virus causes a serious and incurable liver disease and is becoming more prevalent worldwide. This virus is most often transmitted by blood or drug use (needle-sharing) and infection leads to chronic inflammation of the liver and cirrhosis. Hepatitis C infection can develop without symptoms and progress to a chronic stage before people are aware that they carry the virus.

Other causes

Liver failure may also result from overexposure to environmental toxins, such as polyvinyl chloride and carbon tetrachloride, or from complications arising from other diseases, including diabetes mellitus, heart failure and kidney failure.

Diagnosis and Treatment

Symptoms and Diagnosis

Much like skin, blood and bone, the liver is designed to regenerate itself as a response to regular wear-and-tear and injury. At end stage liver failure, no matter the cause, the structure and function of the liver is irreversibly compromised so that the organ is no longer able to regenerate itself. When that happens, patients may experience symptoms such as nausea, vomiting, reduced appetite, jaundice, low-grade fever, abdominal pain and fatigue.

Liver disease is often diagnosed by using liver enzymes as markers of hepatocyte death, or measuring levels of bilirubin (an indicator of normal blood disposal) or abnormalities in blood clotting. Whether liver failure results from acute or chronic causes, end-stage liver disease means that it is only a matter of time before the liver fails.

Treatment

Although the liver can tolerate and recover from extensive abuse, there are often no warning signs that it is failing until it is too late. Once the line is crossed from chronic liver disease to end-stage liver disease or liver failure, the options

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become stark. As of yet, there is no “liver dialysis” that can rehabilitate liver function in the way that kidney failure is treated. Transplantation of a new liver is currently the only effective treatment for liver failure, but it has many drawbacks, including the risk of rejection, risks associated with surgery, and a shortage of donors. It is estimated that for every donor organ there are 10 patients on a waiting list, and many people die from endstage liver disease waiting for a donor organ.

How Can Stem Cells Help?

Using stem cells to treat liver failure is still very much at the experimental stage but the possibility of harnessing stem cells to churn out limitless numbers of hepatocytes for transplant therapy is driving the field forward. As of 2012, adult liver stem cells in humans have not been conclusively isolated so while researchers work towards that goal they are also investigating whether bona fide stem cells from other tissue sources – bone marrow, peripheral blood, fat, skin, amniotic, embryonic and induced – can be used to treat various types of liver failure.

Research directions

Hepatocyte transplants

In their search for novel therapies to treat liver failure, researchers turned to transplanting hepatocytes. It was hoped that this strategy would be able to reverse inborn defects in liver metabolism, bridge patients to whole organ transplantation or even someday replace whole organ transplant. Although dozens of patients with acute liver failure have received hepatocyte transplants from cadaveric donors, with some improvement in liver function, the effects were short lived and there was no overall survival benefit. The major challenges with this approach – shortage of cadaveric donors and immunosuppression of patients – are essentially the same as for whole organ transplants.

Moving forward, researchers are trying to solve the donor problem by learning to grow hepatocytes from different types of stem cells. To do this, they need to identify the signals that specifically stimulate hepatocyte generation and improve their methods for isolating, expanding and storing hepatocytes so that transplanting them can become a viable future therapy for treating liver failure. The expansion issue is not trivial however, because hepatocytes do not easily divide in culture and it is estimated that upwards of 1010 transplanted hepatocytes might be required to see any clinical benefit. The storage issue is also critical because hepatocytes cannot yet be maintained for a long time in culture before they lose their ability to function normally.

Autologous bone marrow stem cells

As far back as 2000, researchers showed that hepatocytes could grow in the body from non-liver cell sources. This phenomenon, called transdifferentiation, was originally observed in female patients who were given bone marrow transplants from male donors: the new liver cells in the female patients could be traced to the donor bone marrow cells from the male donors.

Today, autologous (from the patient) bone marrow stem cells are the only stem cells that have been used clinically for treating liver disease. The major advantage with using these cells is that because they come from the patient, the risk of rejection when they are transplanted back into the patient is very low. In trials spanning 2005 to 2010, autologous bone marrow cells were used as sources of cell therapy for a variety of liver diseases. Most of the trials were small, from one to forty patients and some showed a measure of improvement in liver function.

The way in which bone marrow stem cells actually contribute to liver regeneration is not yet clear. It may be that the cells are transdifferentiating into hepatocytes or perhaps they are producing soluble factors that promote regeneration or repair. There is also the possibility that the stem cells may be fusing with resident hepatocytes to direct their regeneration. The cell fusion mechanism may seem an unlikely but it actually occurs during normal development; for example when myoblasts (muscle cells) form myotubes (muscles) or phagoctyes in the bone marrow form osteoclasts that remodel bone.

In terms of the benefits to the liver, it’s hard to know whether whole bone marrow or particular types of stem cells in

the bone marrow are responsible. Sorted populations such as CD133+ bone marrow cells have been used with some success in patients undergoing removal of extensive portions of the liver but unsorted autologous bone marrow stem cells have also contributed to better liver function in patients with cirrhosis and hepatocellular carcinoma (HCC).

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are found throughout the adult body in tissue such as bone, muscle, cartilage and fat. Other sources for MSCs include bone marrow, cord blood, placenta, fetal liver tissue, and dental papilla. Mesenchymal stem cells are among the most 'multipotent' stem cells that remain in our bodies after birth. This means that they are still able to make a variety of different cell types. Researchers are keenly investigating whether MSCs can transdifferentiate into hepatocytes in vivo or can be coaxed into making hepatocytes ex vivo for transplantation. Preliminary trials have shown that patients with liver cirrhosis have benefitted from autologous bone marrow derived mesenchymal stem cells, but larger, controlled trials will be necessary to predict whether this direction is worth pursuing and how exactly mesenchymal stem cells are exerting their effects.

Mobilizing stem cells with G-CSF

G-CSF is a growth factor that can be used to stimulate CD34+ hematopoietic stem cells to exit the bone marrow and enter the blood. The circulating stem cells can then be easily collected, separated from the other blood cells, and delivered back into the same patients as autologous transplants. Small clinical trials have tested this strategy in patients with end stage liver disease as a result of alcohol or hepatitis B virus-induced cirrhosis. The procedure has been proven safe and in some cases beneficial effects have been observed for as long as 12 months. G-CSF can also be administered alone with the intention of stimulating endogenous liver stem cells in the body to repair damage. Clinical trials are underway to evaluate this strategy as well.

Hepatic stem cells

With adult stem cells having been discovered in many of the body's organs, one might assume that the tremendous regenerative capacity of the liver is due to liver stem cells, but it hasn't been so easy to isolate or characterize these cells in humans. Because normal hepatocytes have an intrinsic capacity to replace themselves many investigators believe that they are the cells that account for how the liver repairs itself. Recently, researchers have identified a liver stem cells in mice and these can produce hepatocytes and other liver cells when transplanted into recipient mice. A similar population of hepatic progenitor cells have been isolated from human liver tissue but so far only in vitro. Unfortunately, pre-clinical studies have thrown up a glitch because human hepatic progenitor cells produce tumours in mice, meaning that much additional work must be done before these cells can be used in a clinical setting.

Artificial livers

Once of the concerns about transplanting cells into a liver undergoing failure is that the environment of the liver may not allow the graft to 'take'. The possibility of creating an artificial liver, similar to a dialysis machine for kidney failure, that would perform all the functions of a normal liver might replace the need for transplants or provide a bridge to therapy for patients waiting for transplants.

Artificial livers loaded with fresh hepatocytes obtained from human or pig livers, have been tested in phase I clinical trials. Despite providing some respite for patients, these artificial liver bioreactors have not provided any overall survival benefit or reduced the need for transplants. In addition, obtaining huge numbers (1010) of fresh hepatocytes to load into the bioreactors is an ongoing issue.

Researchers at the McGowan Institute for Regenerative Medicine in Pittsburg are trying alternate approach where they co-opt part of the body, in this case the lymph node, to act as a reservoir for transplanted hepatocytes. The hepatocytes grow well in the lymph nodes, perhaps because there is a lot of blood supply to the nodes, and the resultant 'hepatasized lymph nodes' are even able to rescue mice with lethal liver failure. One issue with translating this approach to humans is the risk of hepatocyte rejection given the donor population of hepatocytes. Researchers theorize

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that someday perhaps induced pluripotent cells created from the patients themselves could generate the hepatocytes needed for the transplant and thus circumvent the issue of graft rejection.

In an international collaboration, researchers from the USA, Italy and Japan are attempting to create 'neolivers' by reseeding livers stripped down to a three dimensional scaffold with fresh hepatocytes. They have had some success testing this approach in rats and are now optimizing their seeding strategies and making sure that the stripping technique does not compromise the ability of the neoliver to be infiltrated with blood vessels.

Drug testing

Pharmaceutical companies require large numbers of hepatocytes in order to identify which chemicals could be potential new drugs. This procedure is invaluable because 50 per cent of drugs are taken off the market because of toxicity to the liver. Currently, hepatocytes left over from transplants or hepatocyte cell lines derived from liver cancers are used as sources for drug testing but variation and functionality are issues that can confound the results. This is where stem cells could be a tremendous boon. Stem cells could theoretically generate limitless numbers of hepatocytes, and induced pluripotent stem cells could even provide patient-specific hepatocytes to verify that a drug therapy would not harm the patient's liver.

Canadian contributions

In 2010, the Canadian Institute of Health Research devoted $14.8 million to fight chronic liver diseases and organizations such as The Canadian Liver Foundation are tireless in their advocacy and education programs that promote research into the causes, diagnosis, prevention and treatment of liver disease.

In a project funded by the Stem Cell Network, Canadian researchers led by Dr. Gordon Keller have devised new protocols for generating liver cells from either human embryonic stem cells or induced pluripotent stem cells (derived from a patient's skin cells). The experimental liver cells closely resemble normal cells in the human liver and express the types of liver enzymes that are important for metabolizing drugs. Having a ready supply of liver cells for drug testing is important because the ability of liver enzymes to metabolize drugs is different from person to person and thus drug efficacy can vary considerably within the population. Being able to generate liver cells from induced pluripotent stem cells is a tremendous asset and in theory the way such experimental liver cells respond to drugs should mimic the response from the patient's own liver cells. These new protocols are a means to a very important end, which is to perform drug metabolism tests in an effort to predict how people will respond to drug therapies. This information will not only improve the drug discovery process but will also increase the potential for patient-specific therapy in the future.

Clinical studies

Currently there are dozens of open NIH-registered clinical trials investigating stem cells as an intervention for liver disease. The majority of trials are in the phase I/II phase and focus on the safety and/or efficacy the stem cell treatments for diseases such as liver or biliary cirrhosis, liver fibrosis and liver cancer. Particular populations from the bone marrow – such as CD34+, CD133+, endothelial progenitors, mononuclear cells – are most often used as autologous transplants to reduce the chance of the transplant rejection. Mesenchymal stem cells sourced from bone marrow or umbilical cord are also being investigated, and researchers hope that the properties of these cells will work towards regenerating the liver and/or mitigatating inflammation within the organ. There are also a number of trials investigating the potential of the growth factor G-CSF to mobilize autologous stem cells for transplant in patients with liver disease.

As the application of stem cells to treat end stage liver disease moves forward, researchers and clinicians continue to grapple with a number of challenges. The methods for differentiating and scaling up the production of hepatocytes from stem cells is pivotal since huge numbers of the cells will be required for transplants, drug testing, and in vivo models. In addition, although a number of different routes for transplanting stem cells or their progeny into the liver have been tested in humans – through the portal vein, the hepatic artery or splenic artery – the ideal route has yet to be determined. In the case of arterial transplantation, the high pressure in the arteries may contribute to low engraftment, and in patients with cirrhosis of the liver there can be a strange reversal of flow in the portal vein, re-routing

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transplanted cells to the spleen rather than to the liver. Functionality, storage, engraftment and safety are also key issues that will need more work before the full potential of using stem cells to treat end stage liver failure can be achieved.

Web Resources

Readers may wish to peruse the recommended sites below for more information about liver failure and the possible applications of stem cells to treat this condition.

- American Liver Foundation: http://www.liverfoundation.org/
- Canadian Liver Foundation: http://www.liver.ca/
- Health Canada: http://www.hc-sc.gc.ca/ahc-asc/minist/messages/_2011/2011_03_01b-eng.php
- EuroStemCell: http://www.eurostemcell.org/factsheet/chronic-liver-disease-how-could-regenerative-medicine-help

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