Meeting report

Application of stem cells in Gastroenterology and Hepatology, 6th January 2011; In Memory of Dr. Saeid Kazemi Ashtiani, A Great Scientist Who Passed Away Too Soon But Left A Legacy

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Dr. Saeid Kazemi Ashtiani, founder and first president of Royan Institute was born in 1961 in Tehran. In 1993 he started his postgraduate education in the fields of histology and embryology, and went on to obtain a PhD. In 1991, Dr. Ashtiani established Royan Institute in Tehran, a public, non-governmental, non-profit organization, as an infertility clinic. Currently, Royan Institute is a leader in stem cell research and one of the best clinics in the Middle East for infertility treatment. Today, Royan Institute is comprised of three main research departments: Stem Cell Biology and Technology, Animal Biotechnology, and Reproductive Biomedicine.

Dr. Ashtiani believed in the application of science through generation of science to help human beings. Unfortunately, he passed away in 2006 at the age of 44 from heart failure. He opened new horizons in research and scientific experimentation in Iran as well as establishing modern methods of leadership in the scientific community. He was an elegant patriot who loved all nations and humanity as well. May our Lord bless him and he rest in eternal peace.

Therefore in honor of his memory, each year a cutting edge scientific symposium is presented in stem cell technology, concurrent with the commemoration of the anniversary of his death. This year the “Application of Stem Cells in Gastroenterology and Hepatology” symposium was held in January 2011 and included ten practical topics.

The symposium started with a review on clinical trials of liver disorders in Iran by Dr. Reza Malekzadeh, the Director of DDRC and Professor of Medicine at Tehran University. He explained their experiences in cell-based therapies in Iran. Dr. Nickegbalian, a specialist in multivisceral organ transplantation and Professor of Surgery at Shiraz University, described the importance of cell therapy and regenerative medicine for end stage liver diseases. He also reviewed the surgical issues in this subject and explained the problems of patients on waiting lists.

Dr. Samani subsequently presented his lecture on ethical considerations of cell-based therapy for patients in gastroenterology and hepatology. He is the Director of the Epidemiology and Ethics Department at Royan Institute. Behshad Pournasr, a PhD candidate and Research Assistant at Royan Institute presented his lecture on “hESCs and hiPSCs in Gastroenterology and Hepatology”. The fourth lecturer, Dr. Masoud Vosough, a PhD candidate and Research Assistant at Royan Institute discussed the following topic: “What Should Physicians know in Cell-based Therapy of Liver Disorders?” “Tissue Engineering and Stem cell Technology in Hepatology” was presented by Dr. Piryaei, Assistant Professor at Medical Sciences of Shahid Beheshti University. Mohsen Moslem, a PhD candidate and Research Assistant at Royan Institute described “Animal Models of Liver Disease”.

“GMP Standards in Cell Production for Clinical Application” was explained by Dr. Nasser Aghdami, the Director of the Regenerative Medicine Department at Royan Institute. “The Present Status of Cell Therapy in Gastroenterology” was a topic described by Dr. Bagheri, Assistant Professor of Medicine at Tehran University. Finally, Dr. Mohammadnejad explained the “Future Perspectives of Cell Therapy in Gastroenterology and Hepatology”. Dr. Mohammadnejad is an Assistant Professor of Medicine at Tehran University. This symposium was a great opportunity for young Iranian researchers to become more familiar with the applications of stem cells in medicine.

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Evaluation of Clinical Trials of Stem Cell Therapy for Liver Diseases in Iran

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During the last five years, several animal and human studies have demonstrated that both MSCs and HSCs could be used to treat liver cirrhosis.1,2 The basis for this therapeutic effect is the fact that a diseased liver can recruit migratory stem cells from bone marrow to generate hepatocyte-like cells, either by cell fusion or trans-differentiation. These studies have shown that bone marrow stem cell transplantation can lead to regression of liver fibrosis.

Study 1
Our first pilot study1 on the feasibility of this method included four patients with decompensated cirrhosis who were on the liver transplant waiting list. These patients received, via the hepatic artery, a mean number of 5.25×10⁶ CD34+ (90.5% purity) subpopulation of autologous bone marrow-derived stem cells. These CD34+ stem cells were separated from approximately 200 mL bone marrow aspirated from the iliac crest. The study outcome included liver volume as assessed by computed tomography (CT), change in MELD score and patient Quality of Life (Short Form-36) questionnaire. One of the patients died of hepatorenal syndrome shortly after the procedure and before liver transplantation could have been performed. Development of hepatorenal syndrome was thought to be due to contrast nephropathy.3 The remaining three patients were followed over a six month period and despite a trend towards increased serum albumin (from 30.7 to 33.7 g/dL) and a reduction in the prothrombin time (from 17.8 to 16.1 s) during this period, the mean MELD score increased from 16 at enrollment to 17. After the death of patient four, the study was prematurely terminated.

Study 2
Another pilot study4 on the feasibility of this technique investigated the effects of autologous bone marrow MSCs in four patients on the liver transplantation wait list with decompensated cirrhosis. Bone marrow (80 – 100 mL) obtained from the posterior iliac crest was processed and cultured under appropriate conditions to isolate and expand MSCs. Each patient received a mean of 31.7×10⁶ cells infused into a peripheral vein over 30 min. One patient had autoimmune hepatitis and the three remaining patients had cryptogenic cirrhosis. We monitored baseline characteristics obtained at enrollment and at regular intervals during 12 months of the study. The mean MELD at enrollment was 23 and decreased to 20 by the end of the study. Responses to the Short Form-36 questionnaire showed an improvement in quality of life of all patients (the mean physical component scale increased from 31.4 to 65.2, and the mean mental component scale increased from 36.3 to 65.6). Serial CT showed an increase in liver volume in three patients (mean value of 615 mL at baseline and 866 mL six months after transplantation). We concluded that autologous MSC transplantation through a peripheral vein to be safe and feasible in the treatment of liver cirrhosis. Improvements in liver function tests and MELD scores of some of our patients were promising, but a limitation to this study was the small number of patients enrolled and the lack of a control group in the trial. We also did not track the fate of the infused cells to clarify their putative mechanism of action.4

Study 3
We subsequently designed the first randomized controlled study4 that evaluated the efficacy of autologous BM-MSC transplantation in patients with advanced chronic liver disease to determine the efficacy of MSC transplantation (compared to a placebo group) and track stem cells in their bodies. Thirty patients with decompensated cirrhosis were randomly assigned to either case or control groups. BM of patients in both groups was aspirated. In the case group, MSC was cultured. The mean duration of the culture was three months. The control group had BM aspiration and the cells were cryopreserved until the end of the study. As with the case group, after the presumed interval, the control group had an infusion of 100 mL D5W with vitamin B complex as a placebo. We plan to culture and infuse the cryopreserved BM aspirates into the control group if the results of the study are encouraging. The study follows each patient for one year. So far, we have included almost 30 patients in this study. The design, inclusion and exclusion criteria are as in the phase 1 study, but because of increased facilities for the culture of MSCs, about 400×10⁶ (10⁶) cells have been transplanted in each recipient. Patient follow ups include laboratory tests, a CT volumetric study and liver biopsy. In two patients, 10% of the cultured MSCs were labeled with 111In-Oxine and the cells traced in the patients’ bodies by SPECT images. Immediately after intravenous infusion, the labeled MSCs first accumulated in the lungs, and gradually moved to the liver and spleen after a period of several hours to a few days.5 On SPECT images after 24 hours, the tracer distribution was homogenous throughout the liver and spleen. Region of interest (ROI) analysis in the first patient showed that the percentage of the homing of the cells into the liver (following decay and background corrections, and geometric mean calculation) increased from 2.8% at the second hour to 13.5% by the 10th day post-infusion. The percentages of cells in the liver of the second patient were 0% at the second hour and 13% after 10 days.5

An independent group of hepatologists selected by the DDRC Research Council periodically review the results of interim analysis. The board is empowered to recommend termination of the study based upon safety concerns. An interim analysis for 12 patients and controls who finished the one-year follow-up revealed a numerical, but not statistically significant trend of improvement in albumin, prothrombin time, INR, total bilirubin and platelets in the MSC group compared to the control group. The improvement was mild and more prominent after four months (0.8 mg/dL improvement in total bilirubin; 0.4 g/dL improvement in albumin; 104/μL improvement in platelet counts, and 0.2 unit improvement in INR). Since we observed mild improvements in all of the above-mentioned parameters, it is possible that these improvements are real, although the number of cases for this interim analysis is low (n=12 in each group), and the improvements are not statistically significant.4

Discussion
Our study did not show unequivocal benefits of stem cell therapy in ESLD, but it confirmed the safety of this procedure with some favorable effects on a short-term basis. At least, it may be promising as a bridge to liver transplantation. Other clinical tri-
 als investigating the effect of adult BMSCs in patients with liver disease including the effects of the mobilization of bone marrow cells using granulocyte colony-stimulating factor (G-CSF) were mainly uncontrolled and studied only a small number of patients. There are several important unresolved issues regarding stem cell transplantation in the treatment of cirrhosis. The major challenges are: identifying the stages of liver disease at which stem cells should be used, the best type of stem cells to be infused, the minimum effective number of cells and the best route of administration. Our studies show that autologous MSC transplantation through a peripheral vein is safe and feasible in patients with liver cirrhosis. Improvements in liver function tests and MELD scores of some of our patients are promising. However, further controlled trials with longer duration of follow-up should be performed to better clarify the safety and efficacy of this treatment modality. Our studies also highlight the previously established short-term safety of stem cell transplantation.

There are, of course, some mild common side-effects including pain at the infusion site, low grade fever, nausea, and rash. Major concerns are medium- to long-term adverse effects of this type of therapy including progressive liver fibrosis and the development of hepatocellular carcinoma. There is an accumulating body of evidence concerning the malignant potential and liver fibrogenic ability (hepatic stellate cells and myofibroblasts) of BMSCs, although the exact parent cells have not yet been identified. This raises concerns regarding the long-term safety of stem cell infusions, particularly in decompensated cirrhotic patients with minimal hepatic reserve. Furthermore, it has been suggested that poorly differentiated hepatocellular carcinoma originates from hepatic oval cells and BMSCs. Indeed, the transformation of hepatic oval cells to hepatocellular carcinoma cells has been shown in animal models. Similarly, in vitro data suggest that MSCs may undergo malignant transformation after repeated in vitro culture. Whether these data from animal models could be applicable in human is not still clear, however, this potential long-term risk should be considered.

The only type of malignancy that has been reported to develop following HSC transplantation in humans is donor type leukemia cell therapy but it mainly occurs with a cord blood source of stem cells, which is less differentiated and potentially more prone to transformation than the adult bone marrow or peripheral blood stem cells.

References

Ethical Issues in Stem Cell Research and Cell Therapy
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Introduction
Although science is moving at a rapid pace worldwide, unfortunately ethical and legal studies cannot maintain the pace of advancements in biological sciences. In many situations, ethics and law lag behind scientific growth. Stem cells and their potential ability to cure diseases have brought the theory of using cells as replacements for current treatments. Replacement of damaged cells could be the sole treatment option for some inurable diseases. Stem cell research is an important new domain of biomedical research that has the potential to offer viable therapeutic options for debilitating disease and injury.

Ethical principles
The four principles in medical ethics are: respect for autonomy, beneficence, non-maleficence, and justice. These principles, as introduced by Beauchamp and Childress, are well accepted by the majority of physicians and ethicists, although some differences of opinion exist. On the other hand, there is biomedical research. A well-known chapter in the history of research on human subjects began December 9, 1946 when an American military tribunal opened criminal proceedings against 23 leading German physicians and administrators for their willing participation in war crimes and crimes against humanity. Among the charges were that German physicians conducted medical experiments on thousands of concentration camp prisoners without their consent. As a direct result of this trial, the Nuremberg Code was established in 1948, which states that: “The voluntary consent of the human subject is absolutely essential,” making it clear that subjects should give consent and that the benefits of research must outweigh the risks. In 1964, the World Medical Association established recommendations guiding medical doctors in biomedical research involving human subjects. This declaration has since undergone six revisions (the most recent at the General Assembly in October 2008), growing considerably in length from 11 to 32 paragraphs.

Stem cells
Stem cells are cells that have the potential for self-renewal and ability to differentiate into specialized cell types. Stem cells are found in the early mammalian embryo, at around 5 – 7 days after fertilization, and are capable of giving rise to all different cell types of the organism. These embryonic stem (ES) cells are said to be ‘pluripotent’. Other stem cell sources are the fetus, umbilical
cord blood and tissues of the adult organism where they provide a pool of progenitor cells for the development and renewal of specific tissues, blood and the nervous system. There is evidence that some nonembryonic stem cells are able, under appropriate conditions, to differentiate into cell types other than those of the tissue from which they are isolated, but the degree of their developmental plasticity is not yet clear. Here we present the ethical issues in production, research and application of stem cells in humans, as noted by the cell source.

**Embryonic stem cells**

*Production of ES cell lines*

In order to derive ES cells, the embryo must be destroyed at around 5 – 7 days after fertilization (blastocyst stage) by harvesting cells from the embryo’s inner cell mass. The question is whether this is ethically correct. According to the Catholic point of view, conception is the beginning of human life and a zygote is a human. The beginning of human life and personhood of the human embryo is more religious. Protestants believe that embryonic research can be conducted until the embryo is 14 days old. UK regulations on embryonic research are based upon this concept. Research on embryos before 14 days is permitted in Iranian Ethical Codes devised by Iran’s Ministry of Health. As Iran is an Islamic country, it is necessary to receive input from Islamic clergy. Although there may be some differences between clergy leaders’ decrees regarding embryos, however, the majority consider implantation of the embryo in the uterus as the time of life with respect to the embryo, and from this time abortion is punishable. This punishment increases with growth of the embryo weekly until the ensoulement time which is 120 days of age (according to Shia Muslim interpretation). After this time, the penalty for abortion is the same as a complete human. Some Sunni clergy believe the ensoulement time to be around 50 days of the embryo’s age which is similar to some Jewish ideas regarding ensoulement. However, Protestants, Jews and Muslims accept research on pre-implantation embryos.

There are four major issues regarding the use of ES and their differentiated cells: 1) purification, 2) function, 3) transplant rejection, and 4) culture. It is established that embryonic stem cells can produce tumors when transplanted into the body. Numerous attempts have been undertaken to find a way to purify differentiated cells derived from ES cells, but all were unsuccessful. A concern exists that if unpurified differentiated cells are transplanted, the remaining ES cells could cause tumor production in the recipient. It is impossible to state that a cell produced in the lab is completely the same as the body’s cells. Several tests are needed to confirm that the differentiated cells are “very similar” to human cells. The differences between body cells and transplanted cells may cause a problem in the recipient. In other words, the cells may not function appropriately. Since ES cells are derived from embryos that are genetically different from the recipients, transplant rejection may happen as with organ transplantation. For this reason, therapeutic cloning has been introduced, however, other ethical issues exist which will be discussed. In addition, all cell cultures contain some culture media and every differentiation contains some differentiating factors, of which all can be potentially harmful for humans. Of particular concern are those originating from animals, such as “fetal calf serum” or “feeder cells from mouse fibroblasts”. Their use brings into question the ethical concern of mixing animal and human cells. Although feeder-free and serum-free cultures exist, however, many growth factors whose safety has not been proven are also used. The safety of these materials should be established before they are used for humans.

**Therapeutic cloning**

Therapeutic cloning is defined as the use of nuclear transfer from the somatic cell of a person to make a cloned embryo to produce genetically compatible stem cells for therapeutic applications. Some scientists believe this is not cloning which is logical because no cloned creature is produced. However, most scientists doubt the safety of these cells because of the possibility of genetic damage that can occur in the cloning “reprogramming stage” where cells are reprogrammed by an electrical shock. Others consider the resultant cells “unnatural” and “unsafe”. Proof exists in cloned animals that they are not completely healthy. Thus it is too soon to decide about the use of therapeutic cloning for human diseases. On the other hand, the introduction of induced pluripotent stem (iPS) cells that can produce stem cells from the fibroblasts of a human being which are absolutely compatible to that human, may put aside the idea of therapeutic cloning. Other ethical issues exist with the use of therapeutic cloning, such as the production of oocytes. Many ethicists believe that it is acceptable to stimulate the ovaries of a woman to retrieve her eggs solely for reproductive reasons and IVF, however, a woman should not be placed in danger just for “research” purposes.

**Fetal stem cells**

The aborted fetus is a good source of stem cells but it is ethically under question. Use of these cells may encourage illegal abortions in order to obtain to such cells.

**Adult stem cells**

These cells are found in tissues formed after the fetal stage and are limited in their developmental potential by the fact that they only give rise to specific cell types. Thus, they are committed to a specific organ. Many, however, can differentiate into other cells in specific situations. Such cells are mainly located in bone marrow but have been found in adipose tissue, endometrium, and muscle cells. The application of these cells in humans is more or less similar to any other new treatment and should follow the clinical trial process. Since the application of adult stem cells is similar to an autograft, there seems to be less ethical concern in comparison with other methods.

**Cord blood stem cells**

Umbilical cord blood stem cells can be viewed as either embryonic or adult stem cells since they are isolated just after birth and possess differences to both types of stem cells. Therefore they can be classified as a third source of stem cells. The attributes of these cells and their potency to replace the bone marrow of a child brought the idea of cord blood banking which is rapidly growing worldwide. In order to provide cord blood for a good number of patients when taking into consideration the variety of HLAs, it is estimated that at least 50000 units of cord blood should be banked. These cells are not harmful for the mother and baby since this blood is taken from the placenta through the umbilical cord when there is no need for it. Thus, it seems that there is no ethical concern for banking these cells, with the exception of donor autonomy which can be achieved by informed consent. Commercialism of the cord blood bank brought many ethical con-
cerns, but HLA-matched cord blood can rarely be found and the feasibility of such a business is questionable. Therefore the conflict of interest in research and ethical problems for using these cells for humans (priority, need, money, etc.) is less. However, there are several private banks which need to be legally regulated to prevent unethical cellular use. Application of cord blood stem cells for treatment can be followed through clinical trial phases and currently, it can be used for more than 85 diseases.

Clinical trials in cell therapy

There are some differences between cell-based and drug clinical trials. In phase I clinical trials the drug is tested on a few normal volunteers but in cell-based research, it is better to do research on a few volunteers diagnosed with the target disease who are at end stage disease. Follow up plays a key role in cell-based research because the adverse effects of the cells, for example cancer, take time to present. Therefore, it is advisable to go from one phase to another after an appropriate follow-up time. It is important to have long-term follow up of first cases even if it is parallel with other phases of the clinical trials. Thus, at any time a complication is noted the clinical trial can be stopped to evaluate the problem. Other than the above, there is no difference between cell and drug therapy clinical trials.

References


Human Embryonic and Induced Pluripotent Stem (hES and hiPS) Cells in Gastroenterology and Hepatology

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Cell and tissue regeneration in the gastrointestinal tract and its appendix-like liver rely on the existence of stem cells with the self-renewal and multipotency characteristics of these organs. Identifying these stem progenitor cells in the liver and gastrointestinal tract can be a good source for regenerative medicine; however, their low numbers and technical difficulties of isolation are the most important barriers in this field.1,2 Human embryonic stem cells and induced pluripotent stem cells (hES and hiPS cells) have high potential to differentiate into all cell types of an organism including hepatocytes and cells of the gastrointestinal tract; however, their therapeutic application has problems which need additional research to be revealed. Ethical and immunological concerns are the main problems for ES cells.

In this regard, iPS cells favorably bypass these problems, but still are not eligible to be used in regenerative medicine because of their low efficiency production, their tumorigenicity properties, lack of efficient safe protocols for their production and inability to produce functional mature cells in vitro.3

The idea of using pluripotent-derived hepatocytes in regenerative medicine shone when Rambhatala et al. for the first time in 2003, could differentiate ES cells to hepatocytes by adding additional material to their culture media. After several years, Duan et al. (2007) could track the labeled cells in an animal liver by detecting human albumin in serum after transplantation of ES-derived hepatocytes to an animal model of liver disease. In this manner stem cell biologists attempted to find a more efficient protocol to create functional hepatocytes or intestinal cells from pluripotent cells.4 Recently Touboul et al. efficiently differentiated ES cells to hepatocytes by using a chemical approach as well as to fetal hepatocytes which could engrat the liver of an animal model two months post-transplantation.5-6 Spence et al. showed that pluripotent stem cells could differentiate to intestine-like organoids that contained all the cell types of the intestinal organ, including intestinal stem cells. It is promising that iPS-derived hepatocytes could ameliorate liver function in fibrotic and cirrhotic animal models; even they could repopulate the entire liver in an animal model of metabolic disease. This makes them suitable for gene manipulation therapy by transplantation of differentiated iPS cell-derived hepatocytes after gene correction; however, the low efficiency of real mature hepatocyte derivation from iPS cells such as ES cells remains the biggest challenge in stem cell biology and application for curing liver failure. Recently, new reports in direct transformation of a fibroblast to functional cells such as neurons,7 cardiomyocytes,4 melanocytes,8 and blood cells19 by transferring foreign DNA (key regulatory transcription factors) lead to new windows in the field of patient specific research. Although there is no report on direct reprogramming of fibroblasts to hepatocytes or any cells from the gastrointestinal tube; however, this new era of cell fate conversion can be a good tool in regenerative medicine for gastrointestinal diseases in the future.

In summary, pluripotent stem cells with specific characteristics such as their differentiation potential to hepatocytes and intestinal cells, and their ability to improve liver or gastrointestinal disorders in animal models provide several uses in regenerative medicine of gastrointestinal diseases. However, all evidence shows additional benefits for iPS cells in contrast to ES cells, which make them ideal for patient specific cell therapy.

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Application of stem cells in Gastroenterology and Hepatology

What Should Physicians Know in Cell Therapy of Liver Diseases?

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From one point of view, chronic liver diseases which lead to irreversible pathological changes in a healthy hepatic tissue are categorized in two main etiologic groups, hereditary, and acquired. Hereditary disorders in normal physiologic functions of the liver are characterized according to single or multiple genetic abnormalities and their impact on the central metabolic organ. Harmful accumulation of midstream substrates in metabolic pathways is the hallmark phenomena of these anomalies that lead to local irritation of healthy cells. Acquired liver pathologies due to viral infections or improper life-styles and harmful habits may also cause chronic inflammation in the liver. Liver fibrosis and cirrhosis are the final outcomes of constant irritation and chronic inflammation.

Orthotopic liver transplantation (OLT) is the only practical clinical treatment for patients suffering from end stage liver diseases. Limited numbers of donors in addition to the increasing records of potential recipients in waiting lists of whole organ transplantation (WOT) have led researchers to find a better alternative for OLT.¹

Currently, cell-based therapy for inborn errors of metabolic pathways is restricted to allogeneic primary hepatocyte transplantation; however, application of pluripotent stem cells or liver specific progenitor cells can be promising alternatives for human primary hepatocytes in the future.²

For end stage liver disease, cell-based therapy is more complicated and problematic due to irreversible alterations in liver architecture and a proper hepatocyte microenvironment. The core hypothesis in randomized clinical trials (RCTs) applying autologous HSCs and BM-derived MSCs in patients with decompensated chronic liver disease is the paracrine effect of these healthy cells on surrounding environment and consequently shifting the delicate balance from fibrogenesis to fibrolysis.

Regenerative medicine in liver disorders

The concept of cell-based therapy in regenerative medicine inspired potential promise for individuals suffering from chronic and degenerative diseases. Although this application of stem cell technology is occasionally exaggerated in the treatment of various kinds of congenital and acquired disorders, the majority of researchers in this cutting edge of science believe in its wonderful ability for the replacement of lost cells and ability to heal damaged organs.

Self-renewal and pluripotency in differentiation into vast types of dissimilar cells are two core characteristics of stem cells, enabling them for reconstruction of damaged tissues. These two features provide numerous cells with remarkable diversity.

Different types of stem cells

Embryonic (ES) and fetal stem cells, as well as adult stem cells were familiar terms to physicians at the time when induced pluripotent stem cells (iPSC) were introduced to the scientific community. ES cells are obtained from the inner cell mass (ICM) of blastocysts prior to implantation and have the ability to produce any tissue and organ in the body. Ethical issues and tumorigenesis, in addition to immunologic rejection of transplanted cells are crucial prohibitory factors in the clinical application of ES cells despite the fact that they have higher differentiation plasticity in comparison with other stem cells.

The idea of personalized medicine in regeneration of damaged organs was revolutionized when Yamanaka (2006) launched iPSCs. This outstanding finding can enable researchers to replace injured cells and reconstruct ruined organs without an unwanted immune reaction. Regarding the production protocols of iPSCs via viral transduction, the tumorigenic potential of these cells still remains the major limiting and threatening factor, therefore production of safe iPSCs is a concern.¹

Adopt stem cells are naturally located in different organs and continually renew their timeworn specific tissues. Hematopoietic stem cells (HSCs) and bone marrow derived mesenchymal stem cells (BM-derived MSCs) are the most available type of adult stem cells and seem to be a reliable option in cell-based therapy. Autologous transplantation of HSCs has been conducted in various pathological circumstances and its safety has been supported by several scientific publications. Consequently, researchers are now convinced of the clinical feasibility of this new approach.

Cord blood and amniotic fluid cells are examples of fetal stem cells, although the cell population and characteristics of cord blood stem cells are the same as BM-derived stem cells. Ethical considerations and risk of malignancy in these cells are inconspicuous so they have been applied in some clinical therapies, in particular for blood disorders of children. However, due to their limited capacity for differentiation and inadequate cell content to treat adult patients, their broad clinical application needs more knowledge through better research and further clinical experimentations.

Clinical experiments

According to Thorgeirsson and Grisham (2006), there have been 77 available published reports before August 1, 2005, which studied the capacity of HSCs for the generation of hepatocytes in the liver. Although the expected alteration of HSCs to hepatocyte-like cells (HLCs) is still controversial, the positive effect of these cells on physiologic function of hepatocytes is undeniable.² However, paracrine secretion of cytokines and growth factors are the most plausible mechanisms for promotion of cellular functions in pathological hepatic conditions by HSCs.

A search at the site http://clinicaltrials.gov/ for “cell therapy...
for liver” shows numerous clinical trials ongoing or completed worldwide in this field of study. The magnificent acceptance of the scientific community worldwide to apply stem cell technology for cell-based therapy of liver disorders, in recent years, reflects the fact that this novel clinical translational science will be the main topic in the future of medical research.

In summary, cell-based therapies for both hereditary and acquired hepatic disorders seem to be a practical bridge to buy time prior and offer true hope to OLT for patients on waiting lists for WOT.

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Tissue Engineering and Stem Cell Technology in Hepatology
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Liver tissue engineering with stem cell-derived hepatocyte-like cells provides a promising alternative to liver transplantation in patients with acute and chronic hepatic failure. A serious shortage of liver donors, high cost, the need for immunosuppressive medications and the high risk of transplant rejection remain the major limitations in the field of liver transplantation.

Using cells instead of organs in this setting should permit (i) expansion of cells in an in vitro phase, (ii) genetic or immunological manipulation of cells for transplantation, (iii) tissue typing and cryopreservation in a cell bank and (iv) the ex vivo genetic modification of the patient’s own cells prior to re-implantation.1

The creation of an unlimited source of donor cells for hepatocyte transplantation therapy, liver tissue engineering and pharmaceuti-cal applications may be the result of isolation and expansion of progenitor cells or stem cells. The cells are required to function in the same manner as normal hepatic cells, or differentiate into hepatocyte-like cells that can replace the function of the failed liver. The hepatogenic potential of many types of stem cells from different sources has been previously described and the majority of them used for this purpose.

The clinical impact of tissue engineering depends upon our ability to direct cells to form tissues with characteristic structural and mechanical properties from the molecular level up to organized tissue. In most cases, tissue engineering attempts to recapitulate certain aspects of normal development in order to stimulate cell differentiation and functional tissue assembly. The induction of tissue growth generally involves the use of biodegradable and bio-active materials designed, ideally, to provide a mechanical, physical and biochemical template for tissue regeneration.2

Function and differentiation of liver cells and stem cell-derived hepatocyte-like cells are influenced by the three-dimensional organ architecture. Hepatocytes are attachment-dependent cells and lose their liver-specific functions or die without an optimal normal microenvironment, extracellular matrix composition and cell-cell contacts. The use of polymeric matrices and/or nanofibrillar components permits the three-dimensional formation of a neo tissue and specific stimulation by adequate modification of the matrix surface, which might be essential for appropriate differentiation of transplanted cells. In addition to development of bioartificial liver devices (BAL) for extracorporeal liver support and intracorporeal liver replacement, a concept that combines tissue engineering using three-dimensional, highly porous matrices with cell transplantation could be useful.3

However, the creation and development of a functional liver tissue for transplantation must overcome the limited distance of oxygen diffusion and accessibility to blood plasma. Therefore induction and creation of efficient vascular networks has been one of the fundamental challenges of liver tissue engineering either in vitro, for the transplantation of prevascularized constructs, or in vivo, for cellular organization and transplant incorporation within the implantation site. Vascularization in vitro could maintain cell viability during tissue growth, induce structural organization and promote vascularization into a host tissue upon implantation.

In summary, as the liver is a very complex organ and has dual functions of both metabolism and detoxification through incorporation of heterotypic cell-cell interactions, 3D architecture and perfused flow, it is crucial to consider the choice of cell source and culture condition, type of scaffolds and creation of vascular networks for long-term maintenance of tissue-specific functions required to reconstruct a tissue-engineered liver.

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Animal Models in Studying Liver Diseases
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The liver is a dynamic organ in which quiescent differentiated cells (including hepatocytes) and hepatic resident stem cells (called oval cells in rodents) which have the potential to activate in response to hepatic injury, divide to restore cell shortages resulting from injury. Sudden, fast and large scale hepatic cell death in acute liver diseases and the increased ratio of cell death/cell division in chronic liver diseases make the liver a suitable organ for cell based transplantation therapies that can be used as an alternative treatment for organ transplantation. However, the lack of human hepatocytes and hepatic stem cells, and their technical difficulties which parallel ethical and immunological problems involving the use of different types of stem cells in human research have lead to the development of animal models. A close re-
lationship between preclinical and clinical research in cell-based therapies are crucial to standardize a practical protocol that can be used for human diseases. In order to achieve this, animal models should mimic pathophysiological conditions which exist in human disorders. Several models have been developed for acute and chronic hepatic diseases but most have not been standardized as suitable guidelines for clinical investigations. There are some models available for liver diseases that are acceptable for cell transplantation. These animal models should be designed in a way that the liver, which is the target for cell therapy, should have enough space for transplanted cells. On the other hand, the transplanted cells should have selective advantages over hepatocytes that survive in an injured liver.

Animal models for hepatic diseases are primarily classified into three main groups: 1) toxin-induced models, 2) surgical models and 3) models of hereditary liver defects (spontaneous or genetically manipulated).

Administration of hepatotoxic agents is the simplest way to develop a hepatic animal model. There are several toxins such as carbon tetrachloride (CCL4), D-galactosamine, thioacetamide, and acetaminophen, to name a few that can make acute or chronic liver injuries based on the type of toxin used, animal species, age, dose and route of administration (oral, intraperitoneal, subcutaneous or intravenous) in both large and small animals. CCL4 induces hepatic injury by metabolizing to free radicals in the P450 (a main detoxifying agent in the liver) cycle, causing lipid peroxidation in the hepatocytic cell membrane that leads to extensive hepatic tissue damage. CCL4 have been used in mice, rats and pigs, and its effects are promoted by phenobarbital administration. D-galactosamine is metabolized in the galactose pathway within hepatocytes, and causes intracellular uridine deficiency. It has been used in mice, rats, dogs and rabbits. Thioacetamide is primarily used to induce acute liver failure with encephalopathy. Administration of acetaminophen is very useful in the evaluation and treatment of patients who have attempted to commit suicide by this drug. Although hepatotoxic agents do not make suitable animal models similar to those seen in human diseases, however administration of these agents accompanied by retrorsine that inhibits resident hepatocyte proliferation could be a good approach for liver cell therapy. However, in these models space exists for new cells but there is no selective advantage over surviving hepatocytes. The main challenge would be the adverse effects of toxins on the transplanted cells.

Surgical hepatic models which mostly induce chronic liver injuries can be designed by total or partial hepatectomy, bile duct ligation, portal vein or hepatic artery branch ligation and portocaval shunts (PCS). In bile duct ligation, oval cells and cholangiocytes proliferate and lead to hepatic stellate cell activation (primary cause of fibrosis). In portal vein ligation, an asymmetric blood supply causes atrophy in the affected lobe and hypertrophy in the other lobes. The most widespread surgical model involves excision of 2/3 of the liver, which the remaining liver cells expand to restore the normal liver in approximately one week. However, as with PCS, it does not show the main symptoms of liver failure. The best model in the bio-artificial liver (BAL) is the totally hepatocyte-mimicked animal because in these animals there are rapid serologic and biochemical changes that are normally metabolized by the liver. In addition, any positive tests of liver function during the study would be for the functioning support system. Cells transplanted in liver surgical models can be replaced in space generated by surgery but again there is no selective advantage over resident hepatocytes.

Models of hereditary liver defects created due to mutation or genetic modifications provide valuable information about several congenital enzymatic deficiencies and their gene therapies. These defects are similar to abnormalities that occur in humans, however some have potential for cell therapy. Genetic defects such as the Gunn rat (model for Crigler-Najjar type I) and spf-ash mouse (model of ornithine transcarbamylase deficiency) neither generate space nor selective advantage. In the toxic milk mouse (Wilson disease), Watanabe heritable hyperlipidemic (WHHL) rabbit and dalmatian dogs with hyperuricemia there is space but no selective advantage. The best classical models which serve both space and selective advantages are: i) the deficiency in fumarylacetoacetate hydrolase (FAH) that leads to an accumulation of toxic metabolites in the tyrosine degradation pathway and ii) the uPA (urokinase-type plasminogen activator) mouse model, obtained by transgenesis. It should be mentioned that none of the hepatic animal models entirely mimic human disease, but by combining two or three models (i.e., CCL4, hepatectomy and retrorsine) the pathophysiology may be closer to humans.

A new approach in standardizing animal models that are similar to human liver failure involve the generation of chimeric animals with a humanized liver. Tateno et al., in 2004 could repopulate the uPA+/+ /SCID transgenic mouse liver with a human liver (up to 80%). Espejel et al. have injected normal mouse induced pleuripotent stem cells (iPSCs) in FAH mice blastocysts and generated chimeric mice with normal livers. These protocols could be used for human iPSCs, too.

We can conclude that coordination between the design of animal models and clinical trials can contribute to establishment of a fixed protocol for liver cell therapy.

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Current Good Manufacturing Practice Challenges in Cell Therapy Products

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The pharmaceutical industry is highly regulated with many legal requirements in addition to safety, health and environmental regulations enacted by national, state and local authorities. Manufacturing regulatory requirements based on the Federal Food, Drug...
and Cosmetic Act (FD&C Act) are presented as current Good Manufacturing Practices (cGMPs) and cover various issues that include facilities design, product delivery and validation. The “current” in current Good Manufacturing Practices allows for variable solutions to technical issues as well as allowing expectations and regulatory requirements to change with technology without the need to revise actual regulations. Therefore, the regulations describe what needs to be accomplished rather than how to accomplish.\(^{1,4}\)

For example, the FDA as a federal authority issued cGMP regulations in the Code of Federal Register (CFR) 21 as follows:

21 CFR 210 Current Good Manufacturing Practices in Manufacturing, Processing, Packaging, or Holding of Drugs, General
21 CFR 211 Current Good Manufacturing Practices for Finished Pharmaceuticals
21 CFR 600 Biologics Products, General
21 CFR 820 Quality Systems Regulations

The applicable sections of the regulations for finished pharmaceuticals are found in 21 CFR 211, Subpart C—Buildings and Facilities and 21 CFR, 211 Subpart D—Equipment.

The advantages in basic cell biology, i.e., the identification of cell markers and their relationship to the cell’s functional status, genetic mapping and protein production on a cellular level, and the ability to separate and grow individual cell types make cell therapy more efficient as a live pharmaceutical agent. Cell therapy utilizes a remarkable array of innovative methods and presents numerous scientific, technical and regulatory challenges to produce novel and advanced cell, gene and tissue-based therapies.

Cell and gene therapy products are, in critical respects, different from traditional biotechnology and biopharmaceutical agents. They may be derived from autologous, allogeneic, or xenogeneic sourced tissues. The cells may be extensively selected, genetically modified, activated and expanded \textit{ex vivo} prolonging production time and possibly result in an increased risk of contamination and other adverse events. Cell therapy products unlike biotechnology products, which are most commonly the purified products of cells, are composed of living, functional cells.

These complexities in cell engineering and clinical use of cell therapies have led to an increasingly rigorous regulatory environment beyond existing FDA regulations. For this purpose draft regulations specific for these therapies are defined in FDA-CBER’s “Proposed Approach to the Regulation of Cellular and Tissue-Based Products” (1997).

As the majority of cell therapy products are unique, most should follow the established Investigational New Drug (IND), establishing safety and efficacy in clinical trials, and comply with both Good Manufacturing Practices (GMP) and Good Tissue Practices (GTP) regulations. Exceptions to this are products with only minimal manipulation and homologous cells. Key sections in the IND are chemistry, manufacturing and control processes, including standard operating procedures (SOPs), clinical protocol with appropriate endpoints, stopping rules and dosing justification, and the preclinical section. However pre-IND consultation of pre-clinical testing, cell product manufacturing and characterization can foster early communication between sponsors and new drug review divisions in order to provide guidance on the data necessary to warrant IND submission. All clinical trials that provide assurance of the safety and efficacy of newly developed products must operate in compliance with Good Clinical Practices (GCPs).

Good Clinical Practice Guidelines include the roles to protect human rights as subjects in clinical trials and standards on how clinical trials should be conducted. However, Institutional Review Board approval is necessary to assure the protection of research subjects’ rights.

GMPs are defined as “methods to be used in, and the facilities or controls to be used for, the manufacture, processing, packing, or holding of a drug to assure that such drug meets the requirements of the act as to safety, and has the identity and strength and meets the quality and purity characteristics that it purports or is represented to possess”.\(^{5}\) cGMPs may be divided conveniently into ten elements:\(^{3}\):

1. Production and process controls
2. Personnel management
3. Record-keeping
4. Calibration
5. Validation
6. Error management
7. Standard operating procedures (SOPs)
8. Labeling
9. Quality control and auditing
10. Facilities and equipment

In fact, cGMP controls all processes during drug development and production, but the grade of this control increases in parallel with progress in clinical development. Prevention and detection of contamination, and other aspects of product safety are emphasized in Phase I, while full GMPs and product characterization are targeted in Phase III.

Cell engineering itself is a rapidly changing field with many challenges, so rigorous development and validation of processes and analytical methods is essential to warrant cGMP. Every element of the cell engineering process including reagents, devices, processing methods and analytical methods must be defined, controlled and validated for product optimization.

Mesenchymal stem cells (MSCs) are described as plastic-adherent cells, which are able to differentiate to osteoblastic, adipocytic and chondrocytic cells. Their multipotency, immunosuppressive properties and production of cytokines or growth factors make them important tools in treating immune disorders and in tissue repair. Clinical sources of MSCs are bone marrow, adipose tissue and cord blood. MSCs are separated from these sources and subsequently expanded for two to three weeks before transplantation.\(^{6}\)

Most centers commonly use open systems, such as tissue culture plates, flasks, screw-cap centrifuge tubes and pipettes for MSCs production. Using this system increases the contamination risk. Working in a class A environment inside a class B room is essential, which can increase the cost of production. Thus development efforts should focus on creating a closed-system process in which the product is manipulated in pre-sterilized, disposable, and isolated bag and tubing sets. A closed system is advantageous for GMP manufacturing of cell and gene therapy products.

Bulk production instead of individual production is more compatible with cGMP regulations. However, for this purpose an allogeneic source should be used for cell production. Cord blood derived MSCs are less immunogenic, unlike BM and AT; there is not a unique standardized procedure to produce MSCs from cord blood. Various collection methods result in variable cell yield and viability of the MSCs obtained. This variation introduces significant problems into GMP manufacturing of clinical cell therapy.

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products. Bacterial contamination, xenogenic risk and genomic instability are other issues regarding GMP manufacturing, which require qualification and control. The reagents used in cell engineering must be GMP-manufactured for clinical use. However, many cytokines and monoclonal antibodies that are used for MSCs manufacturing are available only as research-grade reagents and require more extensive quality testing under GMP.

Finally, problems in purity evaluation before product release due to a lack of specific markers and changes in differentiation capacity after several passages are serious issues under GMP production of MSCs for clinical use.

The field of cell therapies is evolving quickly and cultured cells are used in a remarkable range of applications. Despite numerous registered clinical trials (500 trials registered at http://www.clinicaltrials.gov/), no consensus exists on standards for cell engineering processes. The rigorous process control, validation, documentation and quality that are specified by GMPs are essential.

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Cell Therapy in Gastroenterology

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Cell based therapies have received attention for treating diseases that have no definitive therapy, or need treatments with limited sources or dangerous outcomes. As a novel idea this mode of therapy has gained popularity in various aspects of medicine. Numerous basic and clinical researches have been performed or are ongoing in different fields of medicine.

Pre-clinical and clinical trials of cell-based therapies have been conducted in various gastrointestinal disorders.

Crohn’s disease (CD)

This disease is one of the two main types of inflammatory bowel diseases with an incompletely defined etiologic basis and, in some cases, is quite difficult to treat. In these instances CD may significantly impact quality of life, and increase morbidity and mortality. Despite recent progresses in the treatment of CD and production of new, effective medications (i.e., anti-TNF agents), there is still a need for more effective therapies.

Hematopoietic stem cell transplantation following bone marrow ablation treatments for hematologic malignancies in patients with concomitant CD and remission of disease after bone marrow transplantation,1 illuminated the possible role of immune-ablation and subsequent immune modulation on the course of this disease and a hope for the use of bone marrow derived stem cells to treat CD.

Different types of cell populations including hematopoietic stem cells, mesenchymal stem cells, allogenic cells and autologous cell based therapies have been used in different animal and human studies with encouraging results.

There are few studies of the use of adipocytes as sources of stem cells for treatment of refractory fistulous CD with exciting results.2

Celiac disease (CeD)

Celiac disease is an inflammatory enteropathy with gluten as the causative immunogenic agent that needs lifelong maintenance of a gluten-free diet. This disease may be unresponsive to the gluten-free diet and need immune-suppressive therapies. At the extreme, there is refractory CeD with aberrant T-cells and the possibility of developing intestinal T-cell lymphoma. There is a report about the use of autologous hematopoietic stem cell transplantation in refractory CeD with aberrant T-cells in seven patients.3 In this study, high-dose chemotherapy followed by autologous hematopoietic stem cell transplantation was determined to be feasible, safe and might result in long-term improvement of patients unresponsive to current drugs.

There is a case report of successful treatment of CeD in a 15 year old girl with concomitant autoimmune hepatitis and occurrence of aplastic anemia who needed allogeneic hematopoietic stem cell transplantation. Transplantation resulted in the cure of her CeD.4

Gastroesophageal reflux disease (GERD) and fecal incontinence (FI)

These are clinically important gastrointestinal disorders that arise from sphincteric dysfunction. Despite the presence of multiple therapeutic choices, in some refractory cases there is the need for endoscopic or surgical intervention.

One surgical approach is to inject bulking agents into the deficient sphincter to increase the sphincter’s bulk and competency.

There are at least two animal studies that have used skeletal muscle-derived stem cells injected into the sphincteric apparatus. These studies have shown the safety and feasibility of the procedure in animal models and the hope for improvement in sphincteric function.

Bile acid malabsorption

This is a common form of intestinal malabsorption caused by different mechanisms involving changes in the ileum and functional decrease in the ability of enterocytes for the enterohepatic circulation of bile acids.

In an animal model (Lewis rat) ileal stem cell clusters were harvested and used for producing neoileum in a jejunal segment which abolished loss of bile acids in the stool.

In conclusion, some phase I/II clinical trials of cell-based therapies have been conducted in gastrointestinal disorders. However, future placebo controlled trials are needed to confirm the possible therapeutic potentials of these new treatment strategies.
References


Future Prospects of Cell-based Therapies in Gastrointestinal and Liver Disorders

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In recent years, there has been considerable interest on cell-based therapies in gastrointestinal (GI) and liver disorders. In 1999, Petersen et al. reported the differentiation of bone marrow-derived stem cells (BMSCs) into hepatic oval cells. Contribution of bone marrow cells to hepatocytes and epithelial cells was reported in female patients who received bone marrow transplantation from sex-mismatched donors. However, subsequent studies have shown that the majority of the reported plasticity of BMSCs resulted from cellular fusion, not true trans-differentiation. Furthermore, it was suggested that during tissue injury, bone marrow stem cells migrate to the injured organ and may help to ameliorate tissue injury.

This was the basis for subsequent translational and clinical studies of cell-based therapies to treat various GI and liver disorders. Several clinical trials of cell-based therapies have been conducted in recent years in patients with liver cirrhosis, Crohn’s disease or celiac disease.

The type of cells to be infused should be carefully selected before starting a translational or clinical study. In patients with metabolic liver disorders or fulminant hepatic failure (FHF), there is a need to deliver sufficient numbers of fully functional hepatocytes to the disorderered liver. However, in patients with liver cirrhosis the aim of cell therapy is to repair liver damage and regress liver fibrosis.

Several phase I studies have shown the feasibility and safety of bone marrow stem cell transplantation in liver cirrhosis. Published phase II/III clinical trials have not shown an unequivocal benefit of bone marrow cell therapy in liver cirrhosis. It appears that cell-based therapies have transient therapeutic effects in some patients with cirrhosis. Most published trials have shown some improvement in liver function tests following cell therapy, however histologically proven regression of human cirrhosis has not been reported, thus far. Our phase II placebo controlled trial of BM mesenchymal stem cell transplantation is underway (NCT00476060). In the near future, we will have additional evidence to determine if bone marrow stem cells have a therapeutic potential in cirrhosis.

BMSCs may express some liver-specific gene markers in species.


