Experimental Intrasplenic Transplantation of Hepatocyte Progenitor Cells in Rats

Thesis

Submitted For Partial Fulfillment
of MD. Degree in General Surgery

Presented by

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Introduction

Liver transplantation is a life-saving therapy to correct end-stage liver disease.

Despite the successful use of living donors and improvements in immunosupression and antiviral therapy, organ demand continues to outstrip the supply ***(Brown, 2005).***

Because of a serious shortage of liver donors, and as the waiting period for liver transplantation is a difficult time associated with a high rate of morbidity and mortality; an alternative therapeutic approach is urgently needed ***(Brown et al, 2006).***

Transplantation of hepatocytes derived from adult or fetal livers could not prove itself as a candidate for the alternative treatment because the source of such cells is also limited at present ***(Fox et al, 2006).***

A novel and exciting approach could be offered through the current developments in the field of stem cell biology ***(Masson et al, 2004).***

Embryonic stem cells and bone marrow cells have been reported to have the potential to differentiate into multi-lineage cells, including hepatocytes ***(Chinzei et al, 2002).***

However, the clinical application of embryonic stem cells obtained from human fertilized ova harbors serious ethical and legal problems ***(Kakinuma et al, 2003).***

Consequently, other sources of stem cells have been sought. Human umbilical cord blood (UCB) cells proved recently to have many advantages as grafts for cell transplantation because of the immaturity of newborn cells compared with adult cells. It has been reported that UCB contains mesenchymal progenitor cells capable of differentiating into marrow stroma, bone, cartilage, muscle and connective tissue. Few studies have recently demonstrated the ability of UCB cells to differentiate into hepatocyte-like cells and thus could be used as a novel therapeutic option for liver failure ***(Nonome et al, 2005).***

Various anatomical sites for cell implantation such as native liver, spleen, peritoneal cavity, kidney, lung, pancreas and fat pads have been investigated, the spleen is considered the most privileged anatomical site for transplantation; it can entrap a limited but sufficient number of hepatocytes within its sinusoids, a process termed "splenic hepatization" ***(Pilichos et al, 2004).***

Aim of work

The aim of the present work is to study the competence of the UCB as a source of hepatic progenitor cells, the metabolic and histopathological effect of intrasplenic transplantation of these cells in induced liver cirrhosis.

Introduction to Stem Cells

The stem cell is the origin of life. At stated first by the great pathologist Rudolph Virchow, "All cells come from cells."***(Sell, 2004).***

According to Leslie Brainerd Arey, the father of modern embryology, the first recorded attempt to understand the origin of life and the early development of the human was most likely made by Aristotle (384–322 BC).

He recognized the early stages of development in the uterus and apparently was the first to contemplate the basic conflict of whether or not a new individual was formed de novo or was pre-formed in the mother and only enlarged during development ***(Arey, 1974).***

Aristotle deduced that the embryo was derived from the mother's menstrual blood, a conclusion that was based on the concept that living animals arose from slime or decaying matter (a hypothesis known in the middle ages as "spontaneous generation") ***(Arey, 1974).***

This concept was generally accepted for more than 2000 years, until its validity became the major biological controversy of the 19th century.

The hypothesis that life did not arise spontaneously, but rather only from preexisting life ***(omne vivum ex vivo)*** was pronounced by ***Leydig*** in 1855 ***(Sell, 2004).***

Virchow (1855) then extended this to postulate that all cells in an organism are derived from preexisting cells ***(omnis cellula e cellula)***; all the cells of the human body arise from a preexisting stem cell, the fertilized egg. The counterhypothesis

of spontaneous generation was not formally disproved until 1864, when Louis Pasteur performed carefully controlled experiments that demonstrated the failure of microorganisms to grow (corruption) in sterilized broth in vessels having long necks that prevented ambient organisms from entering ***(Debre, 1998).***

At present, the question is posed in the context of the conflict over abortion: "When does life begin?"

According to the principles derived from Leydig, Virchow, and Pasteur, life as we know it neither ends nor begins but is continuous. The adult human, for example, is only one stage in the cycle of human life ***(Sell, 2004).***

Until the 1800s, the dominant hypothesis was that pre-formed individuals resided in the egg or the sperm. This pre-formed individual was called a homunculus. The homunculus in the egg was activated to develop after stimulation with sperm, or, conversely, the homunculus in the male sperm was activated to develop when provided an appropriate environment in the uterus.

By the early 1900s, this concept had been proven to be incorrect; the embryo was shown to be formed by the fertilization of an egg, which developed in the ovary of the female, by fusion with a sperm provided by the male. The product of the union of a sperm with an egg is the primordial totipotent stem cell ***(Sell, 2004).***

Following fertilization, the egg undergoes a process of cell divisions and cell migrations known as ***cleavage.***

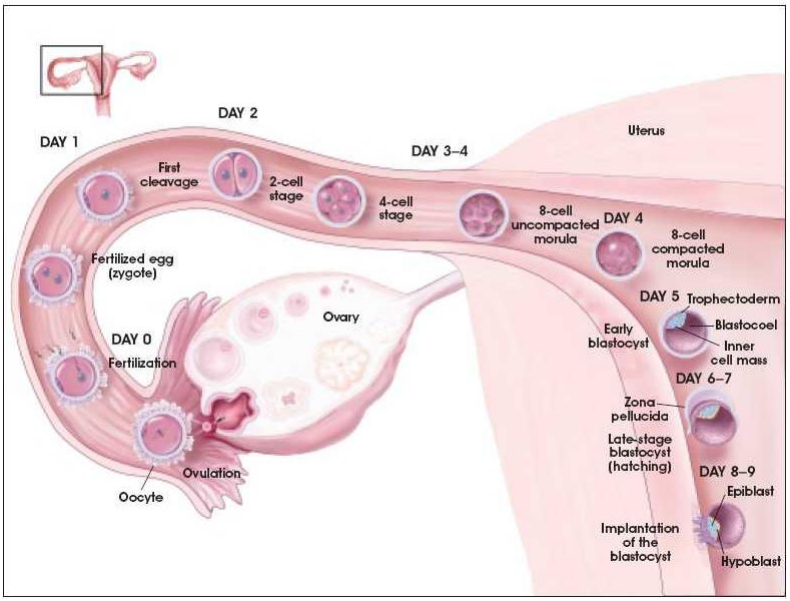

Fig. (1): Development of the Pre-implantation Blastocyst in Humans.

In this early process, each daughter cell receives the full chromosome complement of the original cell, and each daughter cell appears to be the same. This is known as ***symmetric division***, in contrast to the properties of somatic stem cells, which exhibit ***asymmetric division (Merok and Sherley, 2001).***

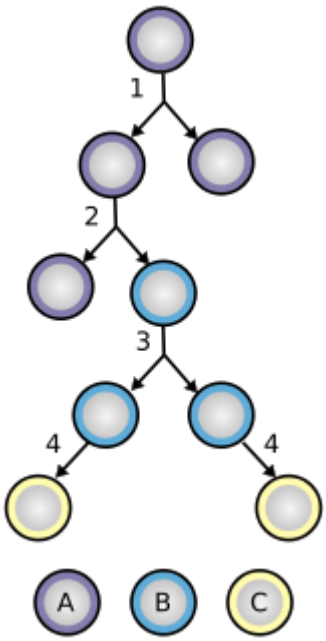

Fig. (2): Stem cell division and differentiation. A - Stem cell; B - progenitor cell; C - differentiated cell; 1 - symmetric stem cell division; 2 - asymmetric stem cell division; 3 - progenitor division; 4 - terminal differentiation

The daughter cells, called ***blastomeres***, stick together to form a cluster of cells known as a ***morula*** (from Morus, mulberry). At each division the blastomeres are reduced in size, but transplantation studies indicate that each embryonic blastomere is able to produce all differentiated cell types; that is, it is ***totipotent (Sell, 2004).***

Eventually, as the number of blastomeres approaches 32 or 64 cells, a cell-free center appears in the expanding cluster of blastomeres, and a hollow sphere of cells is formed ***(blastocyst).*** In mammals, the outer cells form the embryonic membranes and the placenta, whereas the mass of cells within the blastocyst, the inner cell mass (ICM), forms the embryo ***(Sell, 2004).***

At this stage not all the cells are still totipotent, as some of the outer cells become committed to membranes or the placenta. As inner cell mass develops, the daughter cells begin to acquire properties different from one another, so that specific regions are formed that are destined to become different components of the developing embryo, a process known as ***gastrulation.***

During gastrulation, the totipotency of the cells of the inner cell mass is lost, and the blastula is rearranged by invagination of cells from the outer blastocyst to form layered "germ" zones known as ectoderm (outer skin), mesoderm (middle skin), and endoderm (inner skin), which are destined to form the adult organs ***(Sell, 2004).***

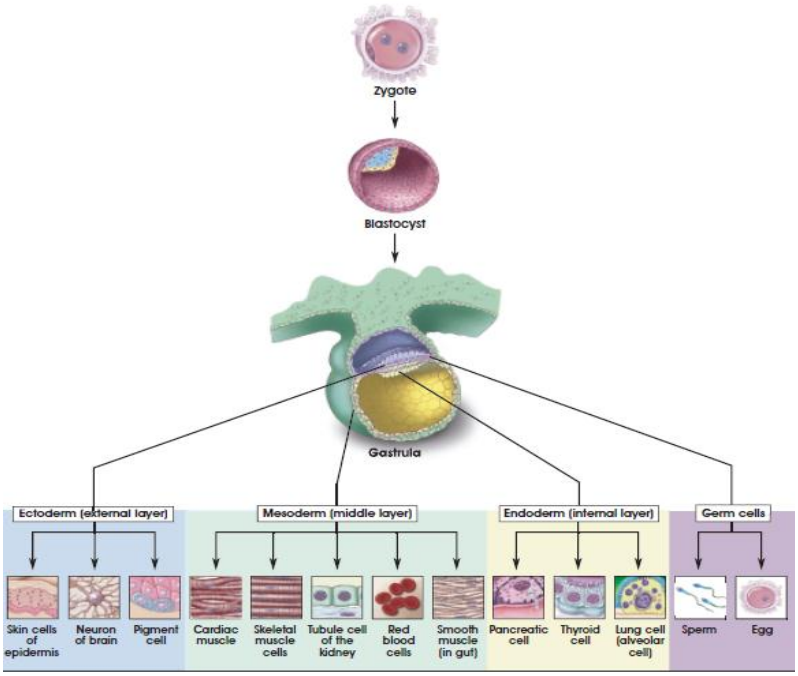

Fig. (3): Differentiation of human tissues

Table (1): Embryonic germ layers from which human tissues develop

EMBRYONIC GERM LAYER	DIFFERENTIATED TISSUE
Endoderm	Thymus Thyroid, parathyroid glands Larynx, trachea, lung Urinary bladder, vagina, urethra Gastrointestinal (GI) organs (liver, pancreas) Lining of the GI tract Lining of the respiratory tract
Mesoderm	Bone marrow (blood) Adrenal cortex Lymphatic tissue Skeletal, smooth, and cardiac muscle Connective tissues (including bone, cartilage) Urogenital system Heart and blood vessels (vascular system)
Ectoderm	Skin Neural tissue (neuroectoderm) Adrenal medulla Pituitary gland Connective tissue of the head and face Eyes, ears

Definitions and Properties of stem cells:

A stem cell is a cell that has the ability to divide (self replicate) for indefinite periods, often throughout the life of the organism.

Under the right conditions, or given the right signals, stem cells can give rise (differentiate) to the many different cell types that make up the organism.

That is, stem cells have the potential to develop into mature cells that have characteristic shapes and specialized functions, such as heart cells, skin cells, or nerve cells ***(Shostak, 2006).***

1- ***Self-renewal:*** the ability to go through numerous cycles of cell divisions while maintaining the undifferentiated state.

2- ***Potency:*** the capacity to differentiate into specialized cell types.

Potency specifies the differentiation potential (the potential to differentiate into different cell types) of the stem cell.

- **Totipotent** stem cells are produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent. These cells can differentiate into embryonic and extra embryonic cell types.

- **Pluripotent** stem cells are the descendants of totipotent cells and can differentiate into cells derived from any of the three germ layers.

- **Multipotent** stem cells can produce only cells of a closely related family of cells (e.g. hematopoietic stem

cells differentiate into red blood cells, white blood cells, platelets, etc.).

- **Unipotent** cells can produce only one cell type, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells).

(Shostak, 2006)

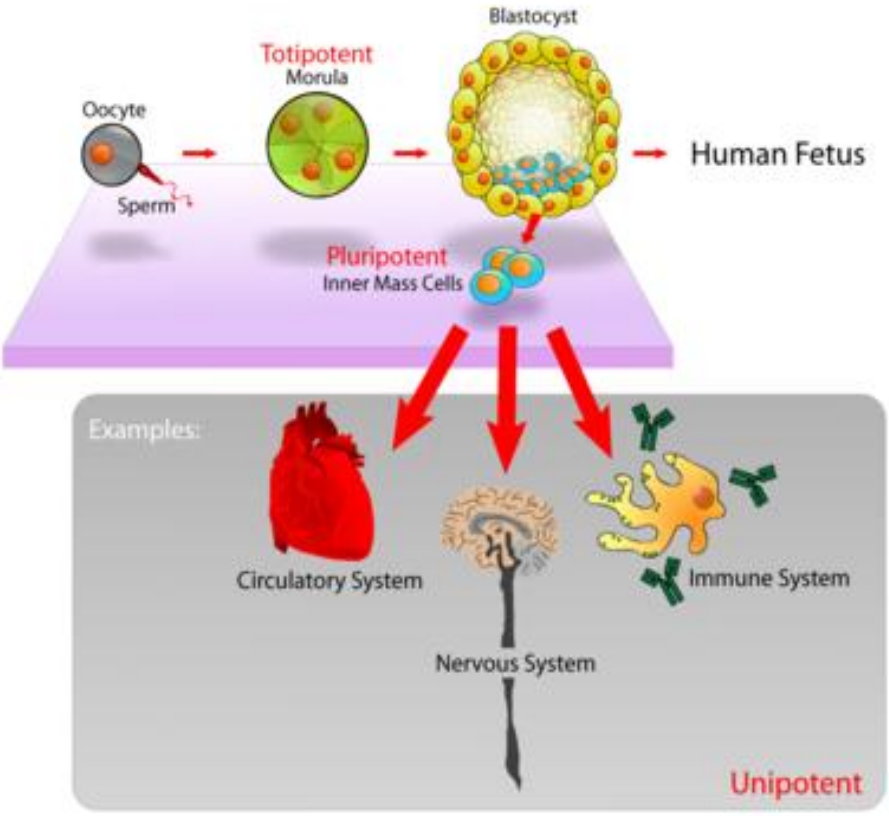

Fig. (4): Potency of human stem cells

Types of human stem cells:

A- Embryonic stem cells:

The embryonic stem cell is defined by its origin, which is from one of the earliest stages of the development of the embryo, called the blastocyst. Specifically, embryonic stem cells are derived from the inner cell mass of the blastocyst at a stage before it would implant in the uterine wall.

The embryonic stem cell can self-replicate and is pluripotent; it can give rise to cells derived from all three germ layers **(*Itskovitz-Eldor et al, 2000*).**

Properties of embryonic stem cells:

- Derived from the inner cell mass/epiblast of the blastocyst.
- Capable of undergoing an unlimited number of symmetrical divisions without differentiating (long-term self-renewal).
- Exhibit and maintain a stable, full (diploid), normal complement of chromosomes (karyotype).
- Pluripotent embryonic stem cells can give rise to differentiated cell types that are derived from all three primary germ layers of the embryo (endoderm, mesoderm, and ectoderm).
- Capable of integrating into all fetal tissues during development. (Mouse embryonic stem cells maintained in culture for long periods can still generate any tissue when they are reintroduced into an embryo to generate a chimeric animal).

- Capable of colonizing the germ line and giving rise to egg or sperm cells.

- Clonogenic that is a single embryonic stem cell can give rise to a colony of genetically identical cells, or clones, which have the same properties as the original cell.

- Expresses the transcription factor Oct-4, which then activates or inhibits a host of target genes and maintains embryonic stem cells in a proliferative, non-differentiating state.

- Can be induced to continue proliferating or to differentiate.

- Lacks the G1 checkpoint in the cell cycle. Embryonic stem cells spend most of their time in the S phase of the cell cycle, during which they synthesize DNA. Unlike differentiated somatic cells, embryonic stem cells do not require any external stimulus to initiate DNA replication.

- Do not show X inactivation. In every somatic cell of a female mammal, one of the two X chromosomes becomes permanently inactivated. X inactivation does not occur in undifferentiated embryonic stem cells.

(*Itskovitz-Eldor et al, 2000)*

Pluripotency of embryonic stem cells:

Pluripotency, that is the ability to give rise to differentiated cell types that are derived from all three primary germ layers of the embryo, endoderm, mesoderm, and ectoderm, is what makes embryonic stem cells unique.

Laboratory-based criteria for testing the pluripotent nature of ES cells derived from mice include three kinds of experiments ***(Odorico et al, 2001).***