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Cell-based therapy for liver diseases

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Abstract. – **Although liver transplantation has become standard therapy in the treatment of patients with liver failure, several problems should be considered in the management of these patients. Other approaches have been proposed, in particular cellular-based procedures. Isolated hepatocytes may be used instead of whole organ transplantation or integrated within the bioartificial devices, in order to replace the missing synthetic and metabolic liver functions. Moreover patient's own hepatocytes may be ex vivo genetically modified to provide the function of a mutant gene. However, new cell sources alternative to adult hepatocytes are actually under investigation, on the basis of recent advances in the field of liver repopulation. Xenogenic primary cells, human hepatoma cells, immortalized hepatocytes and stem cells have been testing in several experiments, even if up to now none of them represent a "gold-standard" for cell-based treatment of liver diseases. In the next future, it is possible that different clinical situations will require different therapeutic approaches, that will be finally defined from the concomitant advances in the development of artificial devices and liver cell biology.**

Key Words:

MARS, Hepatocyte transplantation, Gene therapy, Lliver diseases.

Therapeutic approaches for patients with liver failure

Liver failure may occur as an acute decompensation in patients with chronic liver disease in the presence of a precipitating event or as an acute liver failure in a formerly healthy liver. In both cases, the current standard medical therapy is only aimed to support the patient until the liver spontaneously recovers or a donor is available to perform orthotopic liver transplantation (OLTx)[1].

However, a number of issues in the management of such cases remain to be addressed, in particular organ shortage and poor outcome in patients not supported by liver transplant. In order to overcome these problems, alternative approaches have been proposed, such as auxiliary and split-liver transplantation, procurement from living donors, isolated cell transplantation, transgenic xenotransplantation or extracorporeal liver support[2].

Isolated adult hepatocyte transplantation may represent an important step forward compared to the use of the entire organ, since it is a less invasive and immunogenic procedure and cells from a single donor may be used for multiple recipients. Despite the encouraging results of trials performed in patients with Cliger-Najjar type I syndrome, glycogen storage disease type 1a and ornithine transcarbamylase deficiency, many concerns about the viability of isolated hepatocytes after cryopreservation and the possible formation of cell aggregates during injection remain to be addressed[3-5].

In the context of artificial livers, devices can be distinguish into mechanical devices, able to selectively remove toxins accumulated in the patient's blood and *bioartificial devices*, where isolated hepatocytes are used to simultaneously replace the missing synthetic and metabolic functions of hepatocytes. Up to now bioartificial devices have been demonstrating limited application; so that the Molecular Adsorbents Recirculating System (MARS)[6], consisting of a continuous haemodiafiltration module based on albumin-impregnated permeable membrane, able to remove both protein-bound and

water soluble toxins, represents the most frequently used liver support system[7,8]. Whether there is room for an improvement of albumin-dialysis performance based on the concomitant use of isolated hepatocytes, remains an open question[1].

Finally isolated hepatocytes may be genetically modified in order to provide the function of mutant gene[9]. The main challenge for *gene therapy* is to find a delivery system able to efficiently deliver the therapeutic gene into the target cell. Both viral and non-viral vectors are still under investigation, but none of them actually meets the ideal requirements for liver-directed gene therapy[10].

Liver regeneration and stem cells

Recent publications have demonstrated that liver regeneration is a complex phenomenon involving the proliferation of different cell lineages in response to damage. The first response to parenchimal cell damage is characterized by the rapid hepatocyte-self proliferation, but if a severe and prolonged injury occurs, the liver can call upon endogenous stem cells, namely oval cells. These progenitors are located within the intrahepatic biliary tree and are able to give rise to both hepatocytes and biliary epithelia. Finally, periductular stem cells derived from circulating bone-marrow stem cells may represent the third level of proliferating cells[11].

At this regard, several studies support the evidence that adult haematopoietic stem cell (HSC), removed from their niche into the bone marrow, are able to differentiate acquiring the phenotype and function of other cellular types, such as neuronal cells, pneumocytes, cardiac and skeletal muscle and also hepatocytes (transdifferentation)[12,13].

Focusing the attention on the liver, several experiments demonstrated that bone marrow contains a subpopulation of hepatocyte-oriented stem cells expressing alpha fetoprotein, c-met, CD34 and c-kit[14,15]. The bone marrow-derived hepatocytes were well characterized by Petersen et al in a rat model showing the engraftment of male bone marrow cells into irradiated female recipients, whose livers were damaged by toxic agents in order to obtain a regenerative response[16]. These cells not only present with the phenotype of hepatocytes, but also demonstrate the synthetic and metabolic functions of these cells, as suggested by Lagasse et al in a mouse model of type 1 tyrosinaemia[17]. Similarly, in NOD/SCID mice lethally irradiated and treated with carbon tetrachloride it has been found that bone marrow derived hepatocytes are able to synthesized albumin[18]. Additional experiments performed by our group by treating NOD/SCID mice with allyl alcohol seem to confirm the homing and transdifferentiation of HSC into hepatic cells (Di Campli et al, unpublished data).

In human trials, by using similar gender mismatch transplantation models, several groups have also demonstrated the presence of bone marrow derived cells in the human liver. In particular, Y chromosome positive hepatocytes were identified in livers of female patients previously transplanted with male donor bone marrow and female livers engrafted into male patients[19]. Another study demonstrated that in patients submitted to peripheral blood stem cells transplantation donor-derived hepatocytes can be observed, irrespective of the presence or absence of tissue injury[20].

Therapeutic liver repopulation

To achieve a "therapeutic liver repopulation" the cells used for transplantation should concomitantly be characterized for a high differentiation, a great proliferative capacity and a growth advantage compared to endogenous cells. Experiments performed on animal models suggest that, similarly to the haematopoietic system repopulation occurring after irradiation of bone marrow transplant recipients, the concomitant use of a mitotic stimulus (such as partial hepatectomy or infusions of growth factors) and a cell-cycle block for the endogenous cells (such as irradiation or DNA-damaging agents) may represent the only way to render the hepatocyte transplantation a suitable technique in the clinical setting[21].

The alternative sources of cells actually under investigation, such as xenogenic primary cells, human hepatoma cells, immortalized hepatocytes, arise important infectious, im-

munologic and the carcinogenic concerns in the perspective of the clinical practice. The new insights in the field of liver regeneration have also suggested that a more efficient hepatic repopulation may be achieved by using for transplantation a purified fraction of oval cells, but because of the malignant potential, they should be carefully examined before entering into a clinical transplantation program[22].

This is why the more recent studies have focalized the attention on the use of HSC in the management of patients with liver failure[23]. In fact, these cells may be easily recovered from living donors or umbilical cord blood. The concomitant replacement of both haematopoietic and hepatic systems from the same donor may induce an immunological tolerance, reducing the risk of rejection[24-26]. Moreover, in patients presenting hereditary liver diseases, patient's own HSC could be used to deliver therapeutic genes to the liver, definitively overcoming the main problem of rejection.

However, HSC cannot represent the solution for all the applications of cell-based liver therapy. It is possible that different clinical situations will require different therapeutic approaches and the final answer on what is the best choice for each patient will result from the concomitant advances in the development of artificial devices and in the liver cell biology and gene therapy.

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