

Proceedings of the Annual Symposium on Regenerative Medicine(PASRM)

Cell Therapeutics for Acute Liver Failure Using Foetal Hepatic Progenitors; In Vitro Expansion and In Vivo Implantation in Animal Models

Dedeepiya V^{*1}, Baskar S¹, Parveen N², Khan AA², Habibullah CM², Yoshioka H³, Mori Y³ and Abraham S⁴

¹Nichi-In Centre for Regenerative Medicine, Chennai, India,

²Centre for Liver Research & Diagnostics, Owaisi Hospital, Hyderabad, India,

³Advanced research center for science and engineering, Waseda University, Japan,

⁴Yamanashi University, Faculty of Medicine, Tamaho, Japan.

* Dr. V. Dedeepiya Devaprasad, Nichi-In Centre for Regenerative Medicine, Vijaya Health Centre Premises, 175, NSK Salai, Vadapalani, Chennai-600026, India. E.mail: drddp@ncrm.org

Published online on 26 Dec 2006

Background:

Acute liver failure which affects close to 1,20,000 patients in India every year still lacks a definitive treatment. Though foetal hepatocyte transplantation reported earlier have been successful it is marred with two major obstacles: (i) Lack of timely and adequate quantity of availability of foetal hepatocytes and (ii) short life span of the implanted cells *in situ*. These two problems have been approached by us using a Thermogelation polymer based foetal cell culture technique. Earlier studies have revealed that similar technology and processing method (i) allows *in situ* regeneration of the resected liver portion (Kubota *et al*) and (ii) allows the proliferation of the hepatic progenitors and stem cells into the resected area of the liver *in situ* which form a three dimensional organized structure of the entire damaged portion resulting in normal liver region as before the resection; these two in animal studies(Nagaya *et al*).

Materials & Methods:

I. In vitro study: Hepatocytes were harvested from aborted fetal liver by two-step collagenase digestion method. The cells (5×10^6) were cultured in Mebiol Gel, a thermoreversible gelation polymer gel (TGP) and Matrigel in the same ratio. Once the gel gets solidified 1 ml of DMEM was added and the incubated at 37°C in 5% CO₂ incubator. The cells were observed every day for 7 days. The growth parameters in terms of proliferation, functional capability of the cultured cells viz., ureagenesis, albumin secretion and formazan formation were analysed. II. In vivo animal study: Hepatocytes harvested by two step collagenase digestion method of rat liver were embedded in TGP and transplanted intra peritoneally into acute liver failure rat models induced by D-Galactosamine. The efficacy of the cells embedded in Mebiol Gel was studied by assessing blood parameters and histopathological findings at different time points.

Results:

In vitro study revealed that the decrease in AFP and increase in albumin in the cultured cells and the cells cultured in TGP are functionally more active than cells cultured in Matrigel and TGP based culture yielded more number of mitochondria per unit area, proven by the amount of formazan formed. The ureagenesis study demonstrated that the cells are able to detoxify well in cells cultured in Mebiol. In the animal study, the acute liver failure condition reverted back within 3 days of transplantation. All the blood parameters returned back to normal. The survival rate >70% as compared to the positive control where in only D-Gal was given. The histopathological findings revealed that the hepatocytes survived well even after 30 days of transplantation and there was no infiltration of lymphocytes in and around the recovered TGP embedded cells.

Conclusion:

(i) The foetal hepatocytes undergo proliferation and maturation in TGP much better in both quantity and quality when compared to Matrigel and therefore this method can be used for foetal hepatocyte progenitor expansion, which when done in a large scale a possibility of foetal hepatocyte stem cell bank would be feasible. (ii) The intraperitoneal transplantation of hepatocytes embedded in Mebiol Gel resulted in prolonged survival and function of the cells and was able to support acute liver failure in animal models thus giving a hope that when applied in humans, it could successfully provide liver support in severe acute liver failure when transplanted intraperitoneally.