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Isolation and Transplantation of Hepatocytes in an Experimental Model

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Abstract : Background: Orthotopic liver transplantation is an established treatment for patients with severe acute and end-stage chronic liver disease. The shortage of donor organs continues to be the rate-limiting factor for liver transplantation throughout the world. Hepatocyte transplantation is a promising treatment for several liver diseases and can, also, be used as a "bridge" to liver transplantation in cases of liver failure. Aim of the work: This study was designed to develop a highly efficient protocol for isolation and transplantation of hepatocytes in experimental Lewis rat model to provide satisfactory guidelines for future application on humans.Materials and Methods: Hepatocytes were isolated from the liver by double perfusion technique and bone marrow cells were isolated by centrifugation of shafts of tibia and femur of donor Lewis rats. Recipient rats were subjected to sub-lethal dose of irradiation 2 days before transplantation. In a laparotomy operation the spleen was injected by freshly isolated hepatocytes and bone marrow cells were injected intravenously. The animals were sacrificed 45 day latter and splenic sections were prepared and stained with H & E, PAS AFP and Prox1. Results: The data obtained from this study showed that the double perfusion technique is successful in separation of hepatocytes regarding cell number and viability. Also the method used for bone marrow cells separation gave excellent results regarding cell number and viability. Intrasplenic engraftment of hepatocytes and live tissue formation within the splenic tissue were found in 70% of cases. Hematoxylin and eosin stained splenic sections from 7 rats showed sheets and clusters of cells among the splenic tissues. Periodic Acid Schiff stained splenic sections from 7 rats showed clusters of hepatocytes with intensely stained pink cytoplasmic granules denoting the presence of glycogen. Splenic sections from 7 rats stained with anti-α-fetoprotein antibody showed brownish cytoplasmic staining of the hepatocytes denoting positive expression of AFP. Splenic sections from 7 rats stained with anti-Prox1 showed brownish nuclear staining of the hepatocytes denoting positive expression of Prox1 gene on these cells. Also, positive expression of Prox1 gene was detected on lymphocytes aggregations in the spleens. Conclusions: Isolation of liver cells by double perfusion technique using collagenase buffer is a reliable method that has a very satisfactory yield regarding cell number and viability. The intrasplenic route of transplantation of the freshly isolated liver cells in an immunocompromised model was found to give good results regarding cell engraftment and tissue formation. Further studies are needed to assess function of engrafted hepatocytes by measuring prothrombin time, serum albumin and bilirubin levels.

Keywords : Lewis rats, hepatocytes, BMCs, transplantation, AFP, Prox1
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