ISOLATION OF PRIMARY HUMAN HEPATOCYTES FROM CIRRHOTIC LIVER

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Introduction. Chronic degenerative liver diseases are among most complex social, clinical and epidemiological health problems worldwide. This is due to the steady increase in the incidence and mortality of patients with this pathology. Orthotopic liver transplantation is the only way to save the lives of patients with decompensated diffuse and focal lesions of the liver. One-year survival after liver transplantation reaches 60-80%, but more than half of the patients on the waiting list do not survive until operation. In this regard, hepatocyte transplantation could be an option for the patients who are on the waiting list for organ transplantation.

Materials and methods. We obtained liver tissue from fully consenting patients with end-stage liver cirrhosis undergoing liver transplantation at the City Hospital №1. Primary human hepatocytes were isolated from the pieces of the liver segments II/III using the modified two-step enzymatic method developed by Berry and Friends. After mechanical dissociation, filtration and centrifugation the cells were transferred into a sterile glass dish with collagen and cultivated in DMEM with 10% heat inactivated fetal bovine serum, 1% Penicillin-streptomycin,10|ig /ml of transferrin, 10 |ig /ml of insulin, 0.1|ig / ml of dexamethason, 10 |ig /ml of linoleic acid albumin from bovine serum. Viability of the cells was determined by trypan blue analysis.

Results and discussion. One of the difficulties in isolating hepatocytes from cirrhotic liver is digestion. Since the tissue did not reach the required consistence after enzymatic perfusion, the pieces of liver were additionally placed into an enzymatic solution for 20 minutes. To evaluate the efficacy of the isolation, the amount of live cells was counted after the procedures. The number of viable cells obtained was $8x10^6$ cells per ml (30% viability), thus sufficient for further culturing.

Conclusions. Due to the lack of healthy donor livers as the source of hepatocytes, cirrhotic end-stage livers could become an available source of human hepatocytes for pharmacological and toxicological studies and cell therapy. We were the first research group in Kazakhstan to successfully isolate and cultivate human hepatocytes from cirrhotic liver.