0-080
Successful mouse hepatocyte culture with sandwich collagen gel formation

Dongho Choi1, Yong Jin Kim1, Dan Song1, Kyung Yul Hur2, Jae Joon Kim1, Min Hyuk Lee1, Chul Moon1, Jae Young Jang2, Soung Won Jeong3, Chul Hyung Cho1

1Department of Surgery, 2Department of Internal Medicine, Soonchunhyang University College of Medicine, Seoul, Korea; 3Department of Biomedical Engineering, New Jergy Institute of Technology, New Jergy, USA

Background: Hepatocytes are highly differentiated cells that perform many complex functions. There has been considerable interest in the control of growth and differentiation of hepatocytes in vitro. However, cultures of functional differentiated adult hepatocytes have proved difficult to establish. Collagen gel sandwich method, which was developed by Yarmush group, gave specialized polarity by manipulating the extracellular matrix configuration mimicking hepatic sinusoidal structure in vivo. There were many reports about rat adult hepatocytes with collagen gel sandwich methods. Authors addressed the possibility of maintaining functional hepatocytes with mouse adult hepatocytes with the same methods.

Materials and Methods: Hepatocytes were isolated from adult male C57/B6 mouse (Charles River Laboratories, Boston, MA, USA) weighing 20-30g, using modified two-step collagenase perfusion procedure Primary mouse hepatocytes were sandwiched between two layers of collagen to maintain their stable liver specific functions. Hepatocyte culture medium was changed daily. AFP, albumin, AAT, HNF4A, and were stable in collagen gel with low and high concentration of mouse hepatocytes were evaluated with microscopic finding. In both groups, collagen gel sandwich method showed typical bile canaliculi morphology of the cultured mouse hepatocytes for 10 days compared to collagen coated dish culture method. Gene expression of albumin, AAT, HNF4A, and were stable in collagen gel sandwich method, however, collagen coated dish didn’t showed any expression of hepatic genes after 5 days of culture period.

Conclusions: Collagen gel sandwich method for hepatocytes culture is also available and helpful for mouse adult hepatocytes

Keywords: Mouse hepatocyte, Sandwich collagen gel

0-081
Molecular imaging of hepatic stellate cell activity by visualization of hepatic integrin αvβ3 expression with SPECT in rat

Feng Li, Zhengji Song, Jiwyao Wang

Department of Gastroenterology, Zhongshan Hospital Affiliated to Fudan University, Shanghai, China

Background: The key factors in the pathogenesis of liver fibrosis are the activation and proliferation of hepatic stellate cells (HSCs), which express integrin αvβ3 receptors after activation. This study aims to explore the potential of 99mTc-labeled cyclic arginine-glycine-aspartic acid pentapeptide (cRGD) as an SPECT radiotracer to image hepatic integrin αvβ3 expression to reflect HSC activity in fibrotic livers.

Methods: Rat models of liver fibrosis caused by thioacetamide or CCl4 treatment were employed to examine the expression and distribution of integrin αvβ3 during the development or regression of liver fibrosis. The binding activity of radio-labeled cRGD to integrin αvβ3 was assessed in cultured HSCs and hepatic slices. SPECT was performed to determine hepatic integrin αvβ3 expression in rats with different stages of liver fibrosis.

Results: Protein and mRNA levels of integrin αv and β3 subunits were increased with the progression of liver fibrosis, and reduced with the regression of liver fibrosis. The cell type that expressed majority of integrin αvβ3 in fibrotic livers was activated HSCs. The binding of synthetic cRGD to culture-activated HSCs displayed a high receptor coupling affinity and an abundant receptor capacity. 125I-labeled cRGD bound to fibrotic liver slices, and the binding activity was the highest in advanced fibrosis. Intravenously administrated carboxyfluorescein-labeled cRGD was accumulated in fibrotic liver, and the accumulation amount was increased with the progression and reduced with the regression of fibrosis. SPECT imaging study with 99mTc-labeled cRGD as a tracer demonstrated that the radioactivity ratio of liver to heart increased progressively along with severity of hepatic fibrosis.

Conclusion: Imaging hepatic integrin αvβ3 expression using 99mTc-labeled cRGD as a SPECT radiotracer noninvasively distinguished HSC activity in different stages of liver fibrosis in rats.

0-082
Cilostazol attenuates apoptosis induced by ethanol in rat liver

Youn Ju Lee1, Mi-Sun Suh2, Jong-Yeon Kim2, Jong Ryul Eun2

Department of Internal Medicine, Catholic University of Daegu School of Medicine, 2Yeungnam University College of Medicine, Daegu, Republic of Korea

Background: Alcoholic liver diseases are worldwide health problem. However, the effective treatments are limited. Recently, it has been reported that cilostazol, a selective type III phosphodiesterase inhibitor reduced hepatic fat accumulation induced by high fat high cholesterol diet. In addition, cilostazol has shown its protective effects in various injury animal models. In the present study, we examined the effects of cilostazol on ethanol induced apoptosis in rat liver.

Methods: Primary cultured rat hepatocytes were treated with ethanol (100 and 200 mM) for 24 hr in the presence or absence of cilostazol. The cell viability, caspase-3 activity, cleaved PARP and DNA fragmentation were measured by MTS assay, caspase-3 activity assay, western blotting and Hoechst 33342 staining. RAW 234.7 murine macrophages were treated with