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EXPRESSION OF TIMP-1 AND TIMP-2 IN N-NITROSODIMETHYLAMINE INDUCED HEPATIC FIBROSIS IN RATS. Joseph George, Kanazawa Medical Univ, Uchinada Japan; Mikihiro Tsutusmi, Shujiro Takase, Kanazwa Medical Universitry, Uchinada Japan

Tissue inhibitors of metalloproteinases (TIMPs) are a group of enzymes that regulate the activity of secreted matrix metalloproteinases (MMPs) and modulate the proteolysis of extracellular matrix. In order to obtain more information about the relationship between TIMPs and pathogenesis of liver fibrosis we have studied the expression of TIMP-1 and TIMP-2 during the progression of experimentally induced hepatic fibrosis. The liver injury was induced by intraperitoneal injections of N-nitrosodimethylamine (NDMA) in male albino rats of the Wistar strain in doses of 1 mg/100 g body weight for 7 consecutive days. A group of animals were sacrificed on every day during injection and also on days 14 and 21 from the beginning of the experiment. The α-smooth muscle actin (α-SMA) was stained as a marker for activated stellate cells. Both Hematoxylin & Eosin and Massons trichrome staining was used to monitor the degree of hepatic fibrosis. The expression of TIMP-1 and TIMP-2 was studied immunohistochemically. After NDMA injection necrosis was initiated on day 3 and massive necrosis was observed on days 5 and 7. Fibrosis was well developed by day 14 and early cirrhosis was present on day 21. Serum levels of aspartate transaminase (AST) and alanine transaminase (ALT) increased gradually and peaked on day 7. The elevated levels of serum AST and ALT decreased considerably on days 14 and 21 but not attained the normal value. Immunohistochemical staining of α-SMA demonstrated clear staining of stellate cells from day 3 onwards of NDMA administration. The number of α-SMA positive stellate cells increasedmaximum on day 7, but decreased on days 14 and 21 except the fibrotic zone. Increased staining of both TIMP-1 and TIMP-2 was observed in NDMA induced fibrosis. The staining was more prominent in fibrotic areas. The results of the present investigation demonstrated increased expression of TIMP-1 and TIMP-2 in NDMA induced hepatic fibrosis and early cirrhosis which favors the accumulation of collagen in the liver during fibrosis.

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EFFECT OF AMILORIDE AND/OR PROPRANOLOL ON THE DEVELOPMENT OF COLLATERAL CIRCULATION IN RATS WITH CIRRHOSIS. Jianhua Wang, Frederic Moal, Frederic Oberti, Nary Veal, Vincent Croquet, Paul Cales, Univ, Angers France

Amiloride, a Na+-H+ exchanger inhibitor, has been used as a potassium-sparing diuretic in controlling complication of liver cirrhosis. Chronic propranolol administration is effective in primary and secondary prevention of esophageal varice bleeding. Aim: to study the systemic and splanchnic hemodynamic effects of amiloride and propranolol, alone or in combination, in bile duct ligated rats. Methods: 40 male Sprague-Dawley rats were allocated into 4 groups: vehicle group (saline 1 mL/day), amiloride group (1 mg/kg/day), propranolol group (75 mg/kg/day), and amiloride plus propranolol group (same dosages). Drugs were given by gavage for 4 weeks after bile duct ligation. Hemodynamic parameters including mean arterial pressure (MAP), heart rate (HR), portal pressure (PP), cardiac index (CI), and splenorenal shunt blood flow (SRS), were measured. Results: The results are reported in the table: Conclusion: amiloride has beneficial hemodynamic effect by preventing the development of portosystemic collateral circulation without decreasing portal pressure and without systemic effect. Propranolol, in this study of primary prevention, has no effect on portal pressure or collateral circulation blood flow. This confirm our previous results on the percentage of porto-systemic shunt measured by microspheres (J Hepatol 1997;26:167-73).

	Placebo (n=10)	Amiloride (n=10)	Propranolol (n=11)	Amiloride + propranolol (n=9)	p
MAP (mmHg)	99±9	101±7	100±12	101±10	NS
HR (b/min)	427±36	451±26	402±37	380±31*	0.0002
PP (mmHg)	16±2	15±2	17±2	16±1	NS
CI (mL/min/100g)	52±15	57±16	43±15	44±18	NS
SRS (ml/min)	3.2±3.1	0.8±0.8 **	2.8±3.1	0.8±0.5**	0.04

* $p < 0.01$ vs placebo ** $p < 0.05$ vs placebo

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ZINC DEFICIENCY ENHANCES COLLAGEN EXPRESSION IN HEPATIC STELLATE CELLS. Akiko Kojima, Hiroko Ichikawa, Noriko Takami, Food and Nutrition, Human Life Sci, Osaka City Univ, Osaka Japan; Norifumi Kawada, Shuhei Nishiguchi, 3rd Dept of Int Med , Osaka City Univ Med Sch, Osaka Japan; Isao Matsui-Yuasa, Food and Nutrition, Human Life Sci, Osaka City Univ, Osaka Japan

Introduction: Hepatic stellate cells (HSCs) play an important role in the regulation of extracellular matrix homeostasis and they are key effector cells in liver fibrosis. Myofibroblastic transformation of HSCs is characterized by enhanced expression of ∝-smooth muscle action (∝-SMA) and of various types of extracellular matrix. Serum and liver zinc contents have been found to be significantly lower in cirrhotic patients, and abnormal zinc metabolism and tissue zinc depletion are considered to be closely related to liver diseases. Zinc is an essential element for a wide range of biological activities, some of which are closely related to collagen degradation and synthesis. Indeed, collagenase is a zinc metalloenzyme. Some experimental studies have suggested that zinc administration may have some favorable effects on fibrosis, and these effects could be mediated by its action on collagen synthesis and degradation. The purpose of this study was to assess the effect of zinc on collagen synthesis and degradation in HSCs in culture. We also examined the effect on cell proliferation of hepatocytes and HSCs. Methods: Hepatocytes and HSCs were isolated from male Wister rats by collagenase perfusion and pronase/collagenase perfusion, respectively. Hepatocytes and HSCs (4-14 days of primary culture) were replated and incubated for another 24 hrs with or without zinc chelator, 600 uM diethylenetriamine penta-acetic acid (DTPA). One hour after adding the chelator, cultures were supplemented with ZnSO4 to a final concentration of 600 uM. Desmin,∝-SMA and type I collagen (Co I) in HSCs were detected by immunohistochemical techniques. Furthermore, in order to ascertain the results of the staining, the incorporation of labeled proline into collagenase-digestible proteins was measured as collagen synthesis and degradation. [^{3}H]Proline (0.1 uCi/ml), L-ascorbic acid, and -aminopropionotrile were added to the cell culture 24 hrs before cells were harvested and the media were collected and dialyzed, aliquots were incubated with or without a column-purified bacterial collagenase for 2 hrs and precipitated with trichloroacetic and tannic acids, and the radioactivity was measured in the supernatants. As a marker for cell proliferation, the incorporation of [^{3}H]thymidine into DNA synthesis was measured. [^{3}H]Thymidine (1 uCi/ml) was added to the cell culture 2 hrs before cells were harvested and the incorporation of radioactivity into the acid-insoluble fraction was then measured. Results: To examine the link between zinc deficiency and activation of HSCs, immunohistochemical and biochemical analyses were used. Enhanced Col expression was found in HSCs when DTPA was added, but with addition of zinc Col staining was weak. Increase in the incorporation of labeled proline into collagenase-digestible proteins was observed in DTPA treated HSCs, but it was fully reversed by the addition of zinc. To examine the link between zinc deficiency and DNA synthesis the hepatocytes and HSCs were exposed to DTPA. The incorporation of [^{3}H]thymidine into DNA synthesis in both hepatocytes and HSCs was completely inhibited. However, the inhibition was fully recovered by addition of zinc. Conclusions: These findings suggest that 1) proliferation and activation in HSCs is significantly affected in zinc deficiency, and 2) zinc may prevent liver fibrogenesis.

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EXPRESSION OF CONNECTIVE TISSUE GROWTH FACTOR IN THE LIVER OF PATIENTS WITH CHRONIC HEPATITIS C AND IDIOPATHIC PORTAL HYPERTENSION. Hiroyasu Morikawa, Shuhei Nishiguchi, Susumu Shiomi, Osaka city Univ, Osaka Japan; Shuichi Seki, Osaka City Univ Medical Sch, Osaka Japan; Kenji Kaneda, Osaka city Univ, Osaka Japan; Tohru Nakanishi, Masaharu Takigawa, Okayama Univ, Okayama Japan

Introduction: While low in Western countries, the incidence of idiopathic portal hypertension (IPH, also known as hepatoportal sclerosis, noncirrhotic portal fibrosis, or Banti's syndrome) is relatively high in other countries including Japan and India. Although it has been suggested that immunological abnormalities might be related to the development of IPH, its pathogenesis is still unknown. Connective tissue growth factors (CTGF) has mitogenic, chemotactic and cell matrix-inducing function in fibroblasts and recently demonstrated to be expressed in the liver during chronic hepatitis. IPH is pathologically characterized by prominent portal fibrosis. In this study, on the assumption that this factor may be also involved in the pathogenesis of IPH, we investigated its expression in human subjects. Materials and Methods: Wedge biopsy specimens obtained from healthy volunteers and patients with IPH were subjected to the analysis with Atlas cDNA Expression Arrays. Serum levels of CTGF were measured by the sandwich enzyme-linked immunosorbent assay in a blind manner. Peripheral blood was collected from patients with; i) IPH (74 cases), ii) chronic hepatitis C in a fibrosis stage 1with no bridging (CHC-F1, 24 cases), iii) chronic hepatitis C in a fibrosis stage 2 with P-P bridging, (CHC-F2, 24 cases), iv) chronic hepatitis C in a fibrosis stage 3 with P-C bridging (CHC-F3, 24 cases), v) chronic hepatitis C in a fibrosis stage 4 with cirrhosis (CHC-F4, 12 cases) and vi) healthy volunteers (38 cases). Cryosections of biopsy specimens were immunostained with monoclonal antibody against human CTGF. Results: In Atlas cDNA Expression Arrays, CTGF was one of genes which were overexpressed in the liver of IPH. Serum levels of CTGF were 19.1+ 12.5 ng/ml in healthy volunteers. In patients with CHC-F1, F2, F3 and F4, they were 36.1+ 11.7 ng/ml, 31.7+ 7.5 ng/ml, 32.1+ 6.2 ng/ml, and 34.0+ 9.8 ng/ml, respectively, which were significantly higher than the value in healthy volunteers but not significantly different between each other. In patients with IPH, serum CTGF levels were 28.0 + 32.8, significantly higher than the value in healthy volunteers. Among them, 14 patients showed a considerable increase in serum CTGF levels (> control + 2SD). By immunohistochemistry, CTGF was mildly expressed in the interstitial cells around the portal tract in chronic hepatitis C and IPH. Conclusion: CTGF is expressed in the interstitial cells of the portal tract both in chronic hepatitis C and IPH, and is elevated in the serum, suggesting the role in the development of portal fibrosis in these hepatic disorders.