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Effects of raloxifene on portal hypertension and hepatic encephalopathy in cirrhotic rats

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ABSTRACT

Raloxifene, a selective estrogen receptor modulator, has been used extensively for osteoporosis. In addition to the effect of osteoporosis treatment, emerging evidences show that raloxifene affects the vascular function in different tissues. Cirrhosis is characterized with portal hypertension and complicated with hepatic encephalopathy. Portal hypertension affects portal-systemic shunt which leads to hepatic encephalopathy that the vascular modulation might influence severity of hepatic encephalopathy. Herein, we evaluated the impact of raloxifene on bile duct ligation (BDL)-induced cirrhotic rats. The female Sprague-Dawley rats received BDL plus ovariectomy or sham-operation. Four weeks later, rats were divided into 2 subgroups respectively to receive of raloxifene (10 mg/kg/day) or saline (vehicle) for 14 days. On the 43th day, motor activities and hemodynamic parameters were measured. Hepatic and vascular mRNA and protein expressions were determined. The histopathological change of liver was examined. We found that the liver biochemistry, ammonia level and motor activity were similar between cirrhotic rats with or without raloxifene administration. The hemodynamic parameters were not significantly different except that raloxifene reduced portal venous inflow. Raloxifene exacerbated hepatic fibrosis and up-regulated hepatic endothelin-1 and cyclooxygenase 2 protein expressions. In addition, raloxifene modulated the mRNA expressions of endothelial nitric oxide synthase, cyclooxygenase and endothelin-1 in the superior mesenteric artery and collateral vessel. In conclusion, raloxifene aggravates hepatic fibrosis and decreases portal venous inflow in cirrhotic rats without adversely affecting portal hypertension and hepatic encephalopathy. The modulation of hepatic and vascular endothelin-1, endothelial nitric oxide synthase and cyclooxygenase expressions may play a role in the mechanism.

1. Introduction

The paradox of nitric oxide (NO) distribution in the liver, splanchnic and systemic circulation is the most challenging phenomenon in liver cirrhosis and portal hypertension. Emerging evidences show that decreased NO availability and enhanced intrahepatic vasoconstriction are involved in the mechanism of portal hypertension. On the contrary, increased NO production in the splanchnic and systemic circulation leads to extrahepatic vasodilatation and hyperdynamic circulatory status. The increased splanchnic and portal blood flow further elevates portal pressure (Iwakiri, 2014). Indeed, the agent that is capable of increasing hepatic NO availability and decreasing extrahepatic NO overproduction will be the treatment of choice. What makes the situation more complicated is the distinct functioning of endothelin-1 (ET-1), a potent vasoconstrictor that participates in the development and maintenance of portal hypertension (Yanagisawa et al., 1988; Cahill et al., 1998). It has been found that ET-1 induces potent intrahepatic and portal-systemic collateral vasoconstriction (Lee et al., 1992; Chan et al., 2001). Modulation of the intrahepatic and collateral NO and ET-1 activities may reduce portal pressure and attenuate portal-systemic collaterals (Hsu et al., 2016). Since hepatic encephalopathy, a severe complication of liver cirrhosis is significantly

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related to the severity of portal-systemic collaterals, the amelioration of portal hypertension and collaterals may help in the control of hepatic encephalopathy.

Hepatic encephalopathy is a neuropsychiatric disorder that occurs in patients with acute liver failure, liver cirrhosis or marked portal-systemic shunts. In cirrhotic state, portal hypertension gradually develops after progressive liver fibrosis, which is associated with an imbalance of NO production between the collateral vessels and liver (Wiest et al., 2002). The circulatory toxins, especially ammonia, bypass the liver via collateral vessels and enter the central nervous system, which induces the astrocyte inflammation, brain edema and exacerbates hepatic encephalopathy (Wijdicks, 2016). Recent evidences also indicate that brain neuroinflammation participates in the development of hepatic encephalopathy (Wright et al., 2016).

Raloxifene, a well-known selective estrogen receptor modulator, has been clinically used for the treatment of osteoporosis (Delmas et al., 1997). Raloxifene is a nonsteroidal benzothiophene which exerts estrogen-agonistic effects on bone and lipid metabolism and estrogen-antagonistic effects on uterine endometrium and breast tissue (Ohmichi et al., 2005). In addition to treating osteoporosis, raloxifene has been documented to enhance endothelial NO synthase (eNOS) in the coronary arteries of rabbit via an estrogen receptor-dependent pathway (Figtree et al., 1999). Chronic treatment with raloxifene restores acetylcholine-induced coronary dilatation and preserves endothelial function in ovariectomized hamsters (Chan et al., 2012). Besides, it has been reported that raloxifene at therapeutically relevant concentrations inhibited myogenic constriction via an NO-dependent mechanism (Chan et al., 2010). Furthermore, it has been found that daily oral administration of raloxifene (10 mg/kg/day for 4 weeks) significantly attenuated the increase of right ventricular pressure and pulmonary hypertension of rats, at least in part, by suppressing ET-1 overproduction (Nishida et al., 2009). Taken together, these data suggest that raloxifene is capable of modulating the vascular tone and regional blood flow. However, the relevant effects of raloxifene in cirrhosis with paradoxical hepatic and extrahepatic vascular responses are unknown and deserve further evaluation.

Ozgönül et al. had reported that raloxifene lacked significant influence on the antioxidant enzymes of the liver in normal ovariectomized female rats (Ozgönül et al., 2003.). Furthermore, cirrhotic patients have higher prevalence rates of osteoporosis and fractures (Nakchbandi, 2014) and this has also been proved in patients with primary biliary cirrhosis (Guañabens et al., 2010). A pilot study further revealed that raloxifene improved bone mass in osteopenic women with primary biliary cirrhosis (Levy et al., 2005). Therefore, the animal model with common bile duct ligation (BDL) causes cholestatic liver injury mimicking biliary cirrhosis, is likely to yield the most clinically relevant results. In this study, we thus evaluated the effect of raloxifene on hemodynamic change and hepatic encephalopathy of rats with BDL-induced cirrhosis.

2. Materials and methods

2.1. Animal model of liver cirrhosis with or without ovariectomy

The female Sprague-Dawley rats weighing 240–270 g at the time of surgery were used for experiments. Rats with secondary biliary cirrhosis were induced by BDL under ketamine anesthesia (100 mg/kg, intramuscularly) (Franco et al., 1979). A high yield of secondary biliary cirrhosis was noted after four weeks of the operation (Cameron et al., 1958). Bilateral ovariectomy or sham operation was performed simultaneously upon the time of BDL operation (Wong et al., 2006).

2.2. Study design

The female Sprague-Dawley rats were divided into two groups to receive BDL plus ovariectomy (OVX) or BDL plus sham operation (Sham). Four weeks post the operation, the BDL+OVX rats and BDL +Sham rats were randomly divided into 2 subgroups to receive intraperitoneal injections of raloxifene (10 mg/kg/day) or normal saline (control) for 14 days (Esposito et al., 2005). On the 43th day, measurements of body weight, portal and systemic hemodynamic parameters, degrees of hepatic encephalopathy and blood sampling were performed. Survival was monitored during the period of time. The ammonia, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin were measured at the end of experiments. The mRNA and protein expressions of various molecules were determined in the livers, superior mesentery artery (SMA) and left adrenal veins (the most prominent collateral vessels of cirrhotic rats). The histopathological changes of livers were examined.

The principles of laboratory animal care (NIH publication no. 86-23, revised 1985) were followed. This study was approved by Taipei Veterans General Hospital Animal Committee (IACUC 2012-154).

2.3. Drugs

Raloxifene ([6-hydroxy-2-(4-hydroxyphenyl)-benzothiophen-3-yl]-[4-[2-(1-piperidyl)ethoxy]phenyl]-methanone) was purchased from Eil Lilly and company (Indianapolis, IN, USA). All solutions were freshly prepared on the day of experiments.

2.4. Measurement of motor activities

Motor activities in an open field were determined with the Auto-Track Opto-Varimex activity monitoring system (Columbus Instruments, Columbus, OH, USA). This system is a position tracking system to detect motor activities of small laboratory rodents (Malpass et al., 2010). Rats were housed in a transparent cage (42.2×42.5×20.5 cm). The system utilizes infra-red beams to calculate animal movement and current position. The Opto-Varimex system could be configured by sensor pairs which consist of emitters and detectors. These sensor pairs detect the movements of experimental animals. Distance travelled (cm), resting time (s), ambulatory time (s) and stereotypic time (s) could be recorded. Resting time is equivalent to total time in the chamber minus ambulatory and stereotypic time. During the activity measurements, animals had no access to food or chow. All studies were performed under strictly standardized conditions in the dark room for 30 min. The counting numbers of the total movements, ambulatory movements, and vertical movements were separately recorded to reflect the motor activities of rats with hepatic encephalopathy (Chu et al., 2000).

2.5. Systemic and portal hemodynamic measurements

The right internal carotid artery was cannulated with a PE-50 catheter that was connected to a Spectramed DTX transducer (Spectramed Inc., Oxnard, CA, USA). Continuous recordings of mean arterial pressure and heart rate were performed on a multi-channel recorder (model RS 3400, Gould Inc., Cupertino, CA, USA). The mesenteric vein was cannulated to record the portal pressure.

2.6. Superior mesenteric artery flow and portal venous flow measurements

After carefully dissecting the SMA and portal vein (PV) from their surrounding soft tissue, the SMA and PV blood flow (ml/min/100 mg) were measured using a non-constrictive perivascular ultrasonic transit-time flow probe (lRB, 1-mm diameter; Transonic Systems, Ithaca, NY, USA) placed around the vessel. The flow probe was connected to a small animal flow meter (Transonic Systems).