Methimazole Alleviates Hepatic Encephalopathy in Bile-duct Ligated Cirrhotic Rats

Ching-Chih Chang[1], Yi-Chou Chen[2], Hui-Chun Huang[3,4], Fa-Yauh Lee[2,3,4]*, Full-Young Chang[3,4], Han-Chieh Lin[3,4], Cho-Yu Chan[4,5], Sun-Sang Wang[1,4], Shou-Dong Lee[3,4]

[1]Taipei Municipal Gan-Dau Hospital, Divisions of [2]General Medicine and [3]Gastroenterology, Department of Medicine and [5]Department of Medical Research and Education, Taipei Veterans General Hospital, and [4]National Yang-Ming University School of Medicine, Taipei, Taiwan, R.O.C.

Background: Acute or chronic liver damage may lead to hepatic encephalopathy. Previous studies have indicated the hemodynamic and hormonal mimicry between portal hypertension and hyperthyroidism. Furthermore, medically or surgically induced hypothyroidism has been found to be beneficial in ameliorating hyperdynamic circulation in the portal hypertensive state and in alleviating acute or chronic liver injury in rats. However, the effect of chronic thyroid hormone inhibition on chronic hepatic encephalopathy in cirrhosis remains unknown.

Methods: Liver cirrhosis was induced by bile-duct ligation (BDL) in male Sprague-Dawley rats. Three weeks after BDL, rats were randomized to receive either tap water (control) or 0.04% methimazole in drinking water for 3 weeks. At the end of 6 weeks after BDL, severity of encephalopathy was assessed by the Opto-Varimex animal activity meter and hemodynamic parameters were measured. Blood samples were collected for determination of thyroid stimulating hormone, ammonia and liver biochemistry.

Results: The heart rate of the methimazole-treated group was significantly lower than that of the control group ($p = 0.015$), whereas there were no differences in the mean arterial pressure and portal pressure. The total amount of movements were significantly increased in the methimazole group ($p = 0.029$). Plasma levels of ammonia, aspartate aminotransferase and alkaline phosphatase were significantly lower ($p = 0.01$) and thyroid stimulating hormone significantly higher ($p = 0.035$) in the methimazole group.

Conclusion: Chronic methimazole treatment alleviates hepatic encephalopathy and liver damage in rats with BDL-induced hepatic cirrhosis. [*J Chin Med Assoc* 2006;69(12):563–568]

Key Words: hepatic encephalopathy, liver cirrhosis, methimazole

Introduction

Hepatic encephalopathy (HE) is a complex neuropsychiatric syndrome associated with fulminant liver failure, chronic liver parenchymal disease, or portosystemic shunting.[1–4] The symptom of HE varies, including subtle changes in mentality and alertness, disruptions of physiologic circadian rhythm, or a complete loss of consciousness (hepatic coma). The pathogenesis of HE is not clearly known at present. Numerous substances, such as ammonia, γ-aminobutyric acid, benzodiazepine, aromatic amino acid and false neurotransmitter, have been proposed to be involved in the development of HE.[1–4] The results of previous studies suggest that the pathogenesis of HE could be multifactorial.

It is known that portal hypertension is a hyperdynamic circulatory state characterized by generalized vasodilatation, increased systemic and splanchnic blood flows and increased cardiac output.[5] In fact, the hyperkinetic circulation, hypermetabolism and sympathetic overactivity can also be found in patients with hyperthyroidism.[6,7] Furthermore, in portal hypertensive rats, hypothyroidism induced by methimazole caused amelioration of the hyperdynamic circulation followed by reduction of portal pressure.[8]

In conditions with liver parenchymal injury, propylthiouracil (PTU), a commonly used antithyroid drug,

*Correspondence to: Dr Fa-Yauh Lee, Division of General Medicine, Department of Medicine, Taipei Veterans General Hospital, 201, Section 2, Shih-Pai Road, Taipei 112, Taiwan, R.O.C.
E-mail: fylee@vghtpe.gov.tw • Received: June 30, 2006 • Accepted: October 23, 2006

has been advocated to manage patients with alcoholic liver disease.[9] Hypothyroidism induced medically or surgically even prevented liver cirrhosis in rats that received thioacetamide (TAA) chronically or bile-duct ligation (BDL)[10] and in mice with acute liver injury induced by lectin concanavalin A.[11] Recently, it has been reported that hypothyroidism minimizes liver damage and improves survival in rats with TAA-induced fulminant hepatic failure.[12] However, the impact of chronic thyroid hormone inhibition on chronic HE in cirrhotic status remains to be elucidated. Therefore, this study was conducted in BDL cirrhotic rats with a thyroid hormone synthesis inhibitor, methimazole, to survey the potential of thyroid status manipulation in controlling HE.

Methods

Animal model

Male Sprague-Dawley rats, weighing 240–270 g at the times of surgery were used for experiment. All rats were fasted for 12 hours before operation. A BDL animal model was created as previously described.[13,14] In brief, the rats were anesthetized with ketamine (100 mg/kg intramuscularly) and then the common bile duct was exposed and ligated by 2 ligatures with 3-0 silk. The first ligature was made below the junction of the hepatic ducts and the second ligature above the entrance of the pancreatic ducts. Then, the common bile duct was catheterized by insertion of a PE-10 catheter and 10% formalin (100 μL/100 g) was slowly injected into the biliary tree to prevent the subsequent dilatation of the ligated bile ducts.[15] Finally, the common bile duct was resected between the 2 ligatures. Benzathine benzylpenicillin was administered postoperatively (50,000 U intramuscularly) for prophylaxis of infection. Vitamin K (8 mg/kg intramuscularly) was given after surgery at weekly intervals. The animals were allowed to recover and were studied 6 weeks after surgery. The rats were housed in plastic cages and allowed free access to food and water. In all experiments, the authors adhered to the American Physiological Society Guiding Principles for the Care and Use of Laboratory Animals.

Experimental design

At the end of 3 weeks after ligation surgery, rats with common bile duct ligation were randomized into 2 groups to receive either placebo (tap water, $n=11$) or methimazole (0.04%, $n=12$) in drinking water for 3 weeks. Methimazole was purchased from Sigma Chemical Co. (St Louis, MO, USA). Severity of encephalopathy was assessed at the end of 6 weeks after common bile duct ligation and hemodynamic changes were determined immediately after the assessment of HE. Blood samples were collected for determination of thyroid stimulating hormone (TSH), ammonia and liver biochemistry.

Measurement of motor activities

Motor activities in an open field was determined by using the Opto-Varimex animal activity meter (Columbus Instruments Inc., Columbus, OH, USA).[16–18] The Opto-Varimex activity sensors utilize high-intensity, modulated infrared light beams to detect animal motion. Animals were housed in transparent cages ($17 \times 17 \times 8$ inches) through which 30 infrared beams pass in the horizontal plane, 15 on each axis. This device differentiates non-ambulatory movements (scratching, gnawing) from ambulation on the basis of consecutive interruption of the infrared monitoring beams. An additional row of infrared beams in the horizontal plane (15 on each axis) about 10 cm above the floor was used to count the vertical movements. 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 minutes. The counting numbers of the total movements, ambulatory movements, and vertical movements were separately recorded to reflect the motor activities of rats with fulminant HE.

Hemodynamic measurements

Hemodynamic study was performed under ketamine anesthesia (100 mg/kg body weight, intramuscularly). The right femoral artery was cannulated with a polyethylene PE-50 catheter connected to a Spectramed DTX transducer (Spectramed Inc., Oxnard, CA, USA) and continuous recording of mean arterial pressure was made on a multichannel recorder (model RS 3400; Gould Inc., Cupertino, CA, USA). The external 0 reference limit was placed at the mid portion of the rat. Heart rate was determined from the recording. The abdomen was then opened with a midline incision, and a mesenteric vein was cannulated with a PE-50 catheter connected to a Spectramed DTX transducer. The abdominal cavity was closed and the portal pressure was recorded on a Gould Model RS 3400 recorder.[19,20]

Determinations of plasma TSH, ammonia and liver biochemistry levels

After hemodynamic measurements, the abdomen was opened using a sterile technique. A 3 mL blood sample was collected from the inferior vena cava into a

pyrogen-free syringe containing approximately 75 U of heparin sodium, then placed in an ice bath and transported immediately to the laboratory. Plasma was separated by a refrigerated centrifuge at 4°C and 3,000 rpm for 10 minutes, and then stored at –70°C in pyrogen-free polyethylene tubes for subsequent analysis within 6 weeks. Plasma levels of ammonia and liver biochemistry (including aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase, albumin, total bilirubin) was measured by a Vitro DT chemistry system (Johnson & Johnson Inc., New York, NY, USA) and TSH levels by the ELISA method.

Statistical analysis

Results are expressed as mean ± standard error. Statistical analyses were performed using the paired or 2-sample Student's *t* test when appropriate. Results were considered to be statistically significant when $p < 0.05$.

Results

Hemodynamic changes

Figure 1 shows that heart rates were significantly decreased after methimazole treatment compared to control (methimazole *vs.* control, 214 ± 7 *vs.* 282 ± 19 beats/min, $p = 0.014$). There were no differences in mean arterial pressure (methimazole *vs.* control, 90.0 ± 5.0 *vs.* 99.8 ± 6.0 mmHg, $p = 0.12$) and portal pressure between the 2 groups (17.2 ± 0.8 *vs.* 16.4 ± 0.7 mmHg, $p = 0.436$).

Motor activity count

Figure 2 shows that the total amount of movements was significantly increased in the methimazole group compared with the control group (methimazole *vs.* control, 2,041 ± 106 *vs.* 1,660 ± 123 counts/30 min, $p = 0.029$). Ambulatory (methimazole *vs.* control, 1,206.3 ± 96.7 *vs.* 1,056.5 ± 92.9 counts/30 min, $p = 0.408$) and vertical movements (methimazole *vs.* control, 764.3 ± 100.5 *vs.* 688.8 ± 90.1 counts/30 min, $p = 0.408$) were also higher in the methimazole group, but the differences did not reach statistical significance.

Plasma levels of ammonia, TSH and liver biochemistry tests

The ammonia levels of the methimazole group were significantly lower than those of the control group (97.5 ± 7.5 *vs.* 146.8 ± 14.2 μmol/L, $p = 0.01$). The methimazole group also had significantly lower plasma levels of AST (277.7 ± 44.7 *vs.* 427.5 ± 98.2 U/L, $p = 0.015$) and alkaline phosphatase (317.8 ± 46.0 *vs.* 396.5 ± 56.3 U/L, $p = 0.041$). No significant differences were observed in plasma ALT (190.0 ± 33.7 *vs.* 159.5 ± 19.7 U/L, $p = 0.454$), bilirubin (5.9 ± 0.4 *vs.* 4.6 ± 0.6 mg/dL, $p = 0.09$) and albumin (2.2 ± 0.1 *vs.* 2.6 ± 0.1 g/dL, $p = 0.139$) levels between the 2 groups. The serum levels of TSH in the control group were significantly lower than those in the methimazole group (4.7 ± 0.4 *vs.* 9.9 ± 2.1 ng/mL, $p = 0.035$) (Table 1).

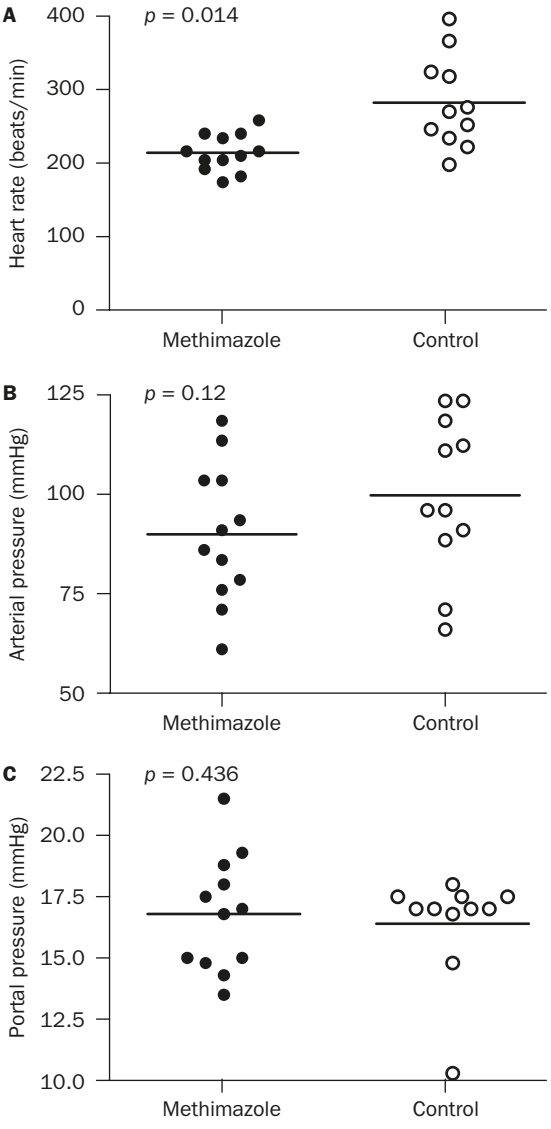

Figure 1. Hemodynamic parameters of methimazole-treated and control groups.

Discussion

The pathogenesis of HE is complicated and not yet fully understood. Common animal models for the study of HE include models of drug-induced fulminant hepatic failure and of portosystemic shunting

induced by various surgical techniques.[1,3,4] Since they represent the 2 extremes of the clinical spectrum of HE, we used another animal model, i.e. BDL rat, to represent chronic liver disease with moderate degree of liver injury and a modest or moderate degree of portosystemic shunting.[13,14] Recently, it has been reported that BDL rats can be regarded as a useful model for studying HE due to liver cirrhosis.[21–23] Indeed, the information provided by this model may be more feasible to be extrapolated to cirrhotic patients with HE.

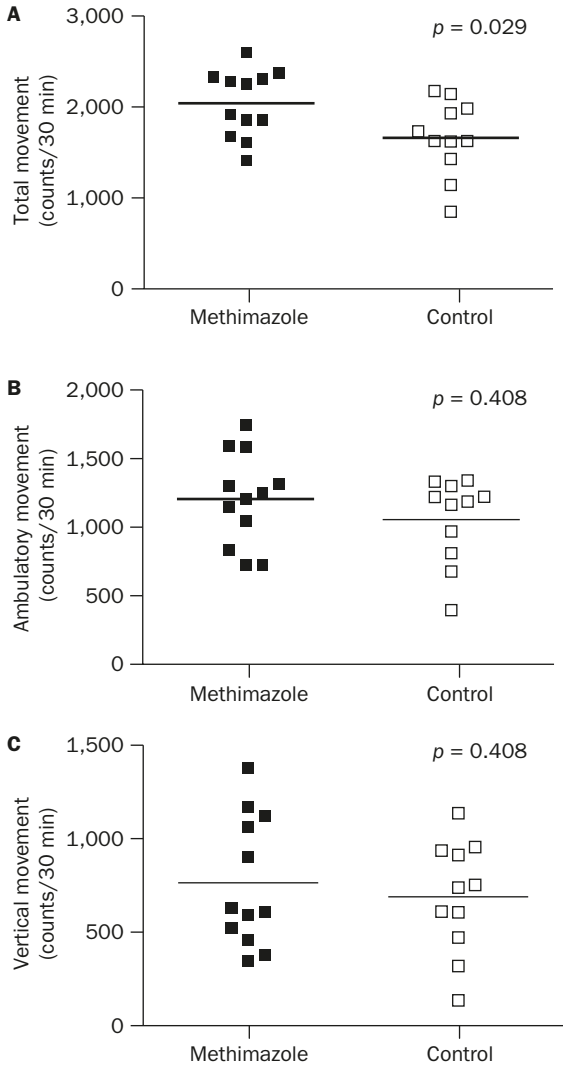

Figure 2. Motor activity counts of methimazole-treated and control groups.

The present study was undertaken to examine whether hypothyroidism that prevents liver damage in several animal models could also be protective in a model of chronic liver disease induced by BDL. Methimazole is 1 of the thioureylene type of antithyroid drugs, an inhibitor of the iodide organification process.[24] In the current study, hypothyroidism induced by methimazole essentially inhibited the development of the ominous manifestations of chronic liver disease, including biochemistry abnormalities and HE. In this study, we also found that the plasma level of TSH in the methimazole group was 2-fold higher than in the control group, compatible with the methimazole-induced hypothyroidism followed by secondary elevation of TSH level.

The mechanisms responsible for the amelioration of liver injury in rats by hypothyroidism are not clear. It has been indicated that hyperthyroidism leads to generalized hypermetabolism and increases hepatocyte oxygen demand. When the condition is not compensated by an increased hepatic blood flow, hepatocyte necrosis ensues, followed by chronic liver damage over time.[25] Immunomodulation might also be responsible, as hypothyroidism inhibits the development of concanavalin A-induced T cell-mediated acute liver damage in mice.[11] In the same study, hypothyroidism adjusted the serum levels of tumor necrosis factor (TNF)-α and interleukin-6 to be near normal in the concanavalin A-treated group. Other studies indicated that in rats and mice, methimazole suppressed the expression of the TNF gene in peritoneal macrophages and reduced alveolar macrophage production under the stimulation of lipopolysaccharide.[26–28] Furthermore, the administration of the soluble receptor of TNF that neutralizes serum TNF-α prevented carbon tetrachloride-induced acute liver injury in rats.[29] The influences can be beneficial, since neutrophils aggravate cholestatic liver injury after BDL.[30] Besides the immunologic factors, some studies pointed out that susceptibility to oxidative stress in mitochondria decreased in hypothyroid status and hypothyroidism offered

Table 1. Plasma levels of liver biochemistry tests and thyroid stimulating hormone (TSH) of methimazole-treated and control groups

	ALT (U/L)	AST (U/L)	ALK-P (U/L)	Albumin (g/dL)	Ammonia (μmol/L)	TSH (ng/mL)
Methimazole	190.0 ± 33.7	277.7 ± 44.7	317.8 ± 46	2.2 ± 0.1	97.5 ± 7.5	9.91 ± 2.13
Control	159.5 ± 19.7	427.5 ± 98.2	396.5 ± 56.3	2.0 ± 0.1	146.8 ± 14.2	4.70 ± 0.44
p	0.454	0.015	0.041	0.139	0.010	0.035

ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALK-P = alkaline phosphatase.

protection against free radical damage.[31,32] In this study, we found higher motor activities and lower plasma AST levels in the methimazole group. We may infer that hypothyroidism induced by methimazole improves the severity of HE in cirrhotic rats, at least partly through the aforementioned mechanisms. Nevertheless, the roles of TNF-α and other proinflammatory cytokines as mediators of liver injury were not determined in the current study.

The use of thyroxine inhibition in the treatment of alcoholic liver disease is based on the finding that the increase in liver oxygen consumption after long-term ethanol administration can be suppressed by thyroidectomy or the administration of methimazole or PTU.[33,34] Oren et al performed a cohort population study of the effects of hypothyroidism on cirrhotic patients.[35] They found a significant improvement in aminotransferase, alkaline phosphatase, albumin, bilirubin and prothrombin time in cirrhotic patients with euthyroidism or subclinical hypothyroidism and concluded that euthyroid patients with liver cirrhosis might benefit from controlled hypothyroidism. Furthermore, Bruck et al found that the level of TAA-induced HE in hypothyroid rats was significantly lower than in euthyroid ones.[12] Nevertheless, some case reports have demonstrated that hypothyroidism may be responsible for hyperammonemia and HE in patients with chronic liver disease.[36,37] The contradictory findings might be associated with the various degrees of hypothyroidism in the different studies.

In conclusion, our current study shows that chronic methimazole treatment improves motor activity and decreases plasma ammonia and AST levels in rats with BDL-induced hepatic cirrhosis. However, caution should be applied in the use of methimazole in the management of HE in patients with liver cirrhosis until more evidence has been obtained.

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