Chronological Production of Thioacetamide-Induced Cirrhosis in the Rat with No Mortality

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Background: *Cirrhotic animal models are useful in studying complications of chronic liver disease. The authors chronologically investigated the effect of thioacetamide (TAA), administered intraperitoneally and adapted individually to weight changes, focusing on the optimal moment to obtain typical features of cirrhosis.*
Material and Method: *Male Wistar Rats,150-200 g, were intoxicated three times per week with TAA of 200 mg/kg for 4, 8, 12 or 16 weeks (n = 8 per group), respectively and compared with age-matched controls (n = 4 per group). The individual body weight and liver function test were also measured in each group. Liver samples from each group were histologically stained with Sirius red in order to identify the degree of liver fibrosis.*
Results: *Rats intoxicated for 4, 8, 12 or 16 weeks had no mortality and histologically showed hepatitis and advanced fibrosis. At 12 and 16 weeks, all animals showed macronodular cirrhosis with signs of high-grade hepatocellular dysplasia. The weight of the treated groups at different time points was significantly lower than the controls. Routine liver function tests between cirrhotic and control rats showed significantly higher only in alanine transaminase (ALT) and aspartate aminotransferase (AST) at 8 and 12 weeks. However, in the cirrhotic rats at 16 weeks, the ALT and AST were much lower than that at 8 and 12 weeks but did not show any difference from the controls.*
Conclusion: *Thioacetamide, adapted to individual weight changes, leads to a model of cirrhosis in the rat at 12 and 16 weeks with zero mortality.*

Keywords: *Thioacetamide, Cirrhosis, Portal hypertension, Rat*

J Med Assoc Thai 2012; 95 (Suppl. 1): S173-S177
Full text. e-Journal: *http://jmat.mat.or.th*

Animal modelsf liver cirrhosis are important for research into the underlying mechanisms or treatments associated with disease. Currently, cirrhosis is mainly induced by the application of hepatotoxins such as carbon tetrachloride (CCl_4) or thioacetamide (TAA)[1]. The cirrhosis model in traumatic and, particular, hepatic fibrosis model may be minimal or absent[2]. In contrast, cirrhosis induced by hepatotoxins, when successful, is stable and irreversible and the unwanted effects of circulating hepatotoxins can be minimized simply by ceasing drug administrations for 1-3 weeks[3,4].

Previous experience has shown that TAA-induced cirrhosis was associated with a relatively lower mortality of 35% compared to 50% with CCl_4-induced cirrhosis (unpublished data). It was also reported that regenerative nodules and liver fibrosis were more prominent in the TAA model than in the CCl_4 model, and that histology of the TAA model was more akin to human cirrhosis[5,6]. In addition, due to the low vaporization of TAA, continuous administration of 0.03% TAA in the drinking water is a convenient and non-invasive method for the induction of cirrhosis in this model[5,7].

In common with other hepatotoxin-induced models, results of the TAA model have been heterogeneous during the stages and development of cirrhosis and this has limited its application. The observation that a given dose of a hepatotoxin could be fatal to one rat but had little effect upon the liver of another rat has indicated the degree of variation in hepatic sensitivity between individual rats[8]. It

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appeared that rats could only become cirrhotic if the doses received were sufficiently high to induce hepatic damage but low enough to avoid death from acute liver failure. The exact magnitude of the safety margin for each individual rat between production of sufficient cirrhosis and death is unpredictable, and the induction of cirrhosis may be often conducted blind if no information regarding hepatic and systemic responses to the toxin is available.

The usual methods of assessing liver damage are by repeated hepatic biopsy and serial blood sampling for liver function tests. These techniques are traumatic and highly dangerous to rats with severe chemical hepatitis or necrosis due to clotting deficiencies. However, Procter and Chatamra[9] described a simple approach to monitor the variation in hepatic responses to intragastric CCl_4 by daily weight changes. In the present study, cirrhosis was induced in rats by TAA with an initial concentration of 200 mg/kg by intraperitoneal injection. The aims of the present study were to determine the optimal range of weight changes that could serve as a guideline for the successful induction of cirrhosis and suitable initial concentration of TAA for induction of cirrhosis by using a modified method.

Material and Method

Animals

Thirty six male Wistar rats weighing between 150-200 g were used in all experiments which were purchased from The National Laboratory Animal Centre (NLAC, Salaya, Thailand). The rats were kept in the room maintained at 25°C on a standardized (12-hour) light/dark cycle and ad libitum. All rats were randomly assigned to the control (no treatment: n = 4) and the treated groups (n = 32). The treated rats were intoxicate three times per week with thioacetamide (TAA) for 4, 8, 12, 16 weeks (n = 8 per group). One rat in the control group and 4 rats in the treated group were collected at the end of the 4^{th}, 8^{th}, 12^{th}, 16^{th} weeks, respectively. The rats in each group were euthanized by carbon dioxide or ether inhalation and killed rapidly by decapitation. The livers were immediately dissected and removed out through a midline abdominal wall incision. The protocol of animal experiment was permitted by the ethic committee from Srinakkarinwirot university by the protocol number 3/2550.

Tissue preparation

The specimens were fixed by an ice-cold mixture of methanol: acetone: water (2:2:1; v/v) at 4°C for 4 hr. Thereafter, each sample was dehydrated in graded series of ethanol and processed till embedded in paraplast. Serial sections of 5-7 μm in thickness were prepared and placed on mounted poly L-lysine-coated slides. The individual body weight and liver function test were also measured in each group. Liver samples from each group were histologically stained with Sirius red in order to identify the degree of liver fibrosis

Results

Mortality, Morphology and Histology

Rats intoxicated for 4, 8, 12 or 16 weeks had no mortality. At 12 and 16 weeks, all animals showed macronodular cirrhosis with signs of high-grade hepatocellular dysplasia (Fig. 1) and histological section showed hepatitis and advanced fibrosis (Fig. 2).

Induction of cirrhosis with minimal weight monitoring

The initial TAA concentration of 200 mg/kg was modified according to the individual variation in hepatic responses to TAA, as monitored by weekly changes in body weight changes. The weight of the treated groups at different time points was significantly lower than the controls (Fig. 3).

Liver function

Routine liver function tests between cirrhotic and control rats showed significant higher only in alanine transaminase (ALT) and aspartate aminotransferase (AST) (Table 1) at 8 and 12 weeks. However, in the cirrhotic rats at 16 weeks, the ALT and AST were much lower than that at 8 and 12 weeks but

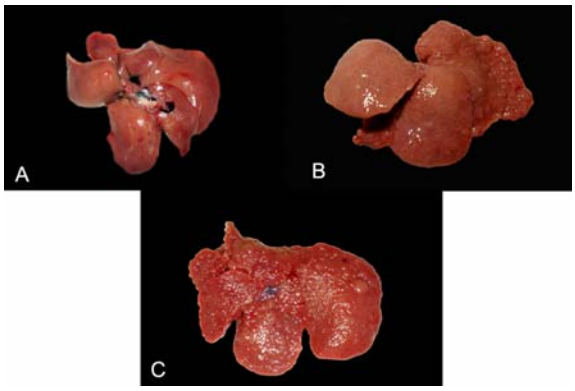

Fig. 1 Macroscopic view of livers from a rat that received 12 weeks (B) and 16 weeks (C) TAA administration. Numerous macronodules can be seen on surface of the cirrhotic liver compared to the normal (A)

did not show any difference from the controls. In addition, small volumes of ascitic fluid (5 ml) were found in 50% (16/32) of cirrhotic rats, similar to the 50% prevalence of ascites in cirrhotic patients[10].

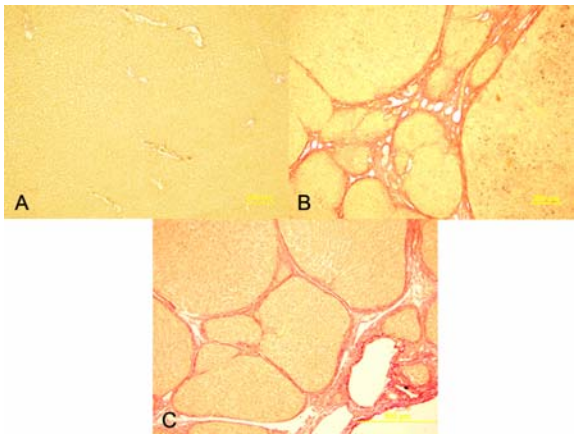

Fig. 2 Histological section of the liver from a rat that received 12 weeks (B) and 16 weeks (C) TAA administration showed fully developed macronodular cirrhosis and advanced fibrosis compared to the normal (A) (Sirius Red x 40)

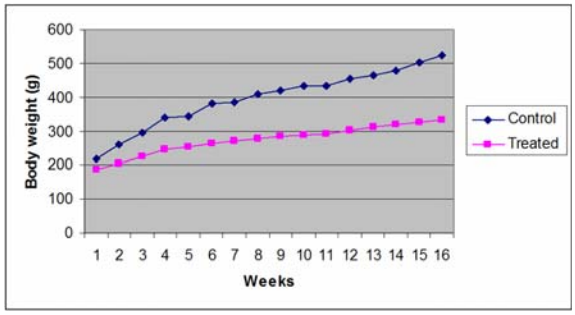

Fig. 3 Body weight changes in responses to concentration of 200 mg/kg TAA administered in the intraperitoneal injection over 16 weeks. Treated groups represented the maximum response (n = 8)

Discussion and Conclusion

In the present study, cirrhosis was induced by concentration of 200 mg/kg TAA administered in the intraperitoneal injection during the development of cirrhosis. This method was effective in the induction of cirrhosis with 50% of rats showing macronodular cirrhosis on macroscopic and microscopic examination. No deaths occurred during induction of cirrhosis.

A previous study demonstrated that the variation and level of critical hepatic damage in response to intragastric CCl_4 could be easily monitored by daily change in body weight[9]. This method was easy to carry out, quick, cheap and without recourse to invasive procedures such as liver function tests, hepatic biopsy, or liver histology that were usually performed in selected rats at fixed time points. In the present study, we used TAA concentration 200 mg/kg for 3 times per week of consecutive 16 weeks.

When TAA concentrations were modified by weight response method during the process of liver damage and development of cirrhosis, 50% of experimental rats finally became cirrhosis with no mortality.

By using this method, well-developed macronodular cirrhosis was obtained after a 16 week administration of TAA. Hepatic histological changes in this model were similar to those found in advanced human cirrhosis where macronodular cirrhosis was usually accompanied with micronodular cirrhosis during a period of approximately 2 years[11].

Acknowledgement

The authors thank Miss Dawrung Srijittapong and Miss Boonma Sripinpao for technical support during animal care.

References

1. Li XN, Huang CT, Wang XH, Leng XS, Du RY, Chen YF, et al. Changes of blood humoral

Table 1. Comparison of differences in routine liver function tests between cirrhotic (8, 12, 16 weeks) and control rats

Group (n)	Total Bilirubin (μmol/l)	AST (lU/l)	ALT (U)	ALP (lU/l)
Control (4)	0.625	117.25	44.50	131.25
Treated 8 weeks (8)	0.900	303.66	108.66	216.33
Treated 12 weeks (8)	0.760	1,196.33	787.66	292.00
Treated 16 weeks (8)	0.930	104.33	42.00	180.66

$p < 0.01$

substances in experimental cirrhosis and their effects on portal hemodynamics. Chin Med J (Engl) 1990; 103: 970-7.
2. Kamath PS, Tyce GM, Miller VM, Edwards BS, Rorie DK. Endothelin-1 modulates intrahepatic resistance in a rat model of noncirrhotic portal hypertension. Hepatology 1999; 30: 401-7.
3. Niederberger M, Gines P, Martin PY, Tsai P, Morris K, McMurtry I, et al. Comparison of vascular nitric oxide production and systemic hemodynamics in cirrhosis versus prehepatic portal hypertension in rats. Hepatology 1996; 24: 947-51.
4. Kaibori M, Kwon AH, Nakagawa M, Wei T, Uetsuji S, Kamiyama Y, et al. Stimulation of liver regeneration and function after partial hepatectomy in cirrhotic rats by continuous infusion of recombinant human hepatocyte growth factor. J Hepatol 1997; 27: 381-90.
5. Zimmermann T, Muller A, Machnik G, Franke H, Schubert H, Dargel R. Biochemical and morphological studies on production and regression of experimental liver cirrhosis induced by thioacetamide in Uje: WIST rats. Z Versuchstierkd 1987; 30: 165-80.
6. Dashti H, Jeppsson B, Hagerstrand I, Hultberg B, Srinivas U, Abdulla M, et al. Thioacetamide- and carbon tetrachloride-induced liver cirrhosis. Eur Surg Res 1989; 21: 83-91.
7. Noda S, Masumi S, Moriyama M, Kannan Y, Ohta M, Sugano T, et al. Population of hepatic macrophages and response of perfused liver to platelet-activating factor during production of thioacetamide-induced cirrhosis in rats. Hepatology 1996; 24: 412-8.
8. Perez TR. Is cirrhosis of the liver experimentally produced by CCl4 and adequate model of human cirrhosis? Hepatology 1983; 3: 112-20.
9. Proctor E, Chatamra K. High yield micronodular cirrhosis in the rat. Gastroenterology 1982; 83: 1183-90.
10. Bosch J, Groszmann RJ. Portal hypertention: Pathophisiology and treatment. Cambridge: Blackwell Scientific, 1994; 4: 45-51.
11. Fauerholdt L, Schlichting P, Christensen E, Poulsen H, Tygstrup N, Juhl E. Conversion of micronodular cirrhosis into macronodular cirrhosis. Hepatology 1983; 3: 928-31.

การชักนำให้เกิดภาวะตับแข็งในหนูด้วยสาร Thioacetamide ในช่วงระยะเวลาที่ต่างกันโดยที่ไม่ทำให้เกิดการตาย

อาทิตย์ นรสิงห์, วิสุทธิ์ ประดิษฐ์อาชีพ, กนกพร ฉายบุระกุล

ภูมิหลังและวัตถุประสงค์: *การศึกษาภาวะแทรกซ้อนของโรคตับเรื้อรัง สามารถศึกษาได้จากสัตว์ทดลองที่ถูกชักนำให้เกิดภาวะตับแข็ง จุดประสงค์ของการศึกษาครั้งนี้เพื่อศึกษาดูช่วงเวลาที่เหมาะสมของสาร thioacetamide ที่ชักนำให้เกิดลักษณะของภาวะตับแข็ง โดยการฉีดเข้าบริเวณชั้นใต้เยื่อบุช่องท้องในช่วงระยะเวลาต่างๆ กัน*

วัสดุและวิธีการ: *ชักนำหนูสายพันธุ์ Wistar rats ตัวผู้น้ำหนักประมาณ 150-200 กรัม ให้เกิดตับแข็งโดยใช้สารเคมี Thioacetamide ฉีดเข้าบริเวณชั้นใต้เยื่อบุช่องท้อง สัปดาห์ละ 3 ครั้ง ในปริมาณ 200 มิลลิกรัมต่อน้ำหนักตัว แบ่งหนูทดลองออกเป็นสี่กลุ่ม คือกลุ่มที่หนึ่งถึงสี่ทำการฉีดสาร thioacetamide เป็นระยะเวลาติดต่อกัน 4, 8, 12 และ 16 สัปดาห์ตามลำดับ (n = 8 ตัวต่อกลุ่ม) และทำการชั่งน้ำหนักและวัดค่าผลการทำงานของตับ (liver function test) ของหนูแต่ละตัว จากนั้นทำการเปรียบเทียบน้ำหนักตัว และผลการทำงานของตับของหนูแต่ละกลุ่มกับหนูกลุ่มควบคุม (n = 4 ตัว) และเพื่อดูระดับของการเกิดพังผืดในตับเนื้อเยื่อของตับในแต่ละกลุ่มจะนำมาย้อมสี sirius red และดูโดยกล้องจุลทรรศน์*

ผลการศึกษา: *หนูที่ถูกชักนำให้เกิดตับแข็ง เป็นระยะเวลา 4, 8, 12 และ 16 สัปดาห์ เกิดลักษณะของภาวะตับแข็งขึ้นและยังมีชีวิตอยู่ได้ และพบว่าหนูทุกตัวในกลุ่มที่ถูกชักนำให้เกิดตับแข็งเป็นระยะเวลา 12 และ 16 สัปดาห์ เกิดเนื้องอกของเซลล์ตับ น้ำหนักตัวของหนูกลุ่มทดลองน้อยกว่ากลุ่มควบคุม อย่างมีนัยสำคัญทางสถิติในแต่ละช่วงเวลาของการทดลอง จากการวัดค่าการทำงานของตับ (liver function test) พบว่าค่า alanine transaminase (ALT) และ aspartate aminotransferase (AST) ของหนูกลุ่มทดลองที่ถูกชักนำให้เกิดตับแข็งเป็นระยะเวลา 8 และ 12 สัปดาห์สูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ แต่อย่างไรก็ตามค่า ALT และ AST ในหนูกลุ่มทดลองที่ถูกชักนำให้เกิดตับแข็งเป็นระยะเวลา 16 สัปดาห์ ต่ำกว่ากลุ่มที่ถูกชักนำให้เกิดตับแข็งเป็นระยะเวลา 8 และ 12 สัปดาห์ แต่เมื่อเทียบกับกลุ่มควบคุมพบว่าไม่มีความแตกต่างกัน*

สรุป: *สาร thioacetamide ที่ฉีดให้กับหนูในปริมาณที่เหมาะสมตามน้ำหนักตัวที่ต่างกันเป็นระยะเวลา 12 และ 16 สัปดาห์ สามารถชักนำให้เกิดตับแข็งได้โดยไม่ทำให้เกิดการตาย*