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Establishment of a liver fibrosis model in cynomolgus monkeys

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ABSTRACT

Hepatic fibrosis, resulted from hepatoxicity and other diseases such as diabetes, is an important pathological characteristic of chronic liver diseases. Establishment of hepatic fibrosis animal models is of great importance and a prerequisite for human clinical studies. The common models for liver fibrosis are often established in lower small animals such as rats, but non-human primates are a much better model for human diseases because of the physiological similarity with humans. In this study, we investigated the method to induce liver fibrosis in cynomolgus monkeys using carbon tetrachloride (CCl_4) and to establish a model that more closely mimics human liver fibrosis. We successfully established the liver fibrosis model in 15 of the 20 cynomolgus monkeys (success rate 75%), by CCl_4 administration at a dose of 1.0 mL/kg (400 mL/L) twice a week. Liver biopsy showed that liver fibrosis progressed with time and gradually advanced into early-stage cirrhosis in 10 of the 15 established models at 16 weeks. Our study provides a better research platform for the prevention and treatment of chronic liver diseases.

Liver diseases are of high morbidity and have become a major health problem and should be addressed urgently (Zhuang, 2009). Hepatic fibrosis is an important pathological characteristic of chronic liver diseases (Ramachandran and Iredale, 2009) and an indispensable developmental stage from chronic liver diseases to cirrhosis. Fibrogenesis progresses gradually and is reversible, whereas advanced cirrhosis is difficult to reverse (Ramachandran and Iredale, 2009; Ismail and Pinzani, 2009). Therefore, timely blockage of chronic liver fibrogenesis is critical to the prevention and treatment of chronic liver diseases (Fallowfield and Iredale, 2004). Establishment of hepatic fibrosis animal models is of great importance and a prerequisite for human clinical studies (Liu and Gao, 2002). Previous liver fibrosis modeling often use small lower animals such as rats, however, non-human primates are a much better model for human diseases because of the physiological similarity with humans. In this study, we successfully induced liver fibrosis in cynomolgus monkeys with carbon tetrachloride, which provides a better research platform for the prevention and treatment of chronic liver diseases.

1. Materials and methods

1.1. Animals

Twenty healthy male cynomolgus monkeys (6–8 years; 6.0–7.0 kg) were provided by the Guangxi Cynomolgus Monkeys Breeding Center and the Guangxi Experimental Animal Center. All animals were individually caged in a room with ventilation, 50–70% of humidity and at 20–29 °C at the Breeding Center. All the procedures were approved by the Medical Ethical Committee and the Laboratory Animal Care Ethical Committee at Guangxi Medical University, Guangxi, China.

1.2. Reagents and liver biopsy instrument

Reagents: Analytical Grade Reagent CCl_4 (≥99.5%) and ethanol were provided by Guangdong Xi-Long Chemical Co., Ltd (China). CCl_4 was mixed in olive oil to 400 mL/L. The liver biopsy gun and semi-automatic biopsy needles (18G L–130 mm) were from TSK Corporation (Japan). Serum aspartate transaminase (AST), alanine transaminase (ALT), total protein (TP), albumin (ALB), and globulin (GLO) values were measured using the Cobas 8000 modular analyzer (Hoffmann-La Roche, US). Four liver fibrosis markers, which include hyaluronic acid (HA), type III pre-collagen (PC III), collagen IV (C IV) and laminin (LN) were measured using MAGLUMI

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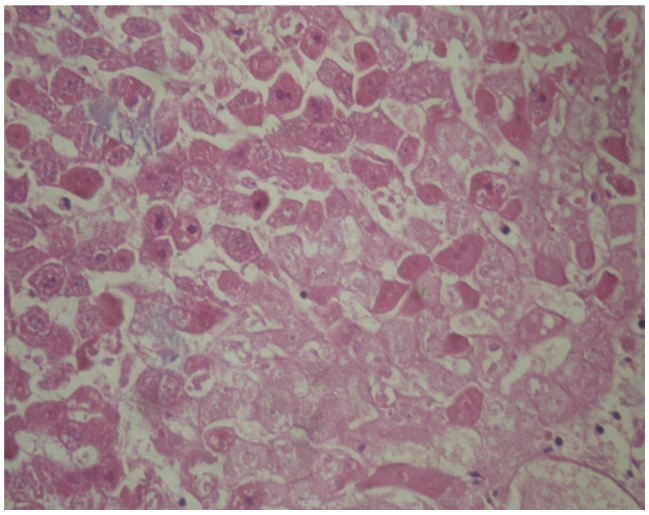

Fig. 1. Pathological changes in liver tissues (H&E stain, 400×). Large areas of dissolved, necrotic hepatocytes are shown. Necrotic hepatocytes are characterized by cell nuclei loss, liver sinusoids disappearance and vanished cell outline was shown for dissolving cells. Remaining hepatocytes revealed eosinophilic changes.

2000PLUS (Shenzhen New Industries Biomedical Engineering CO., LTD, China).

1.3. Hepatic fibrosis model establishment

Cynomolgus monkeys were kept in stainless steel cages and adapted for one week before experiment. Analytical grade CCl_4 was diluted to 400 mL/L in olive oil and was given at 1.0 mL/kg subcutaneously twice a week. The animals were fed with high fat diet containing about 35% of cholesterol (provided by the Breeding Center), and 10% ethanol as the only source of drink. Blood samples were drawn and liver biopsy was performed before and at the 4th, 8th, 12th, 16th weeks after CCl_4 administration on the anterior segment of the right lobe of the liver under ultrasound guidance to avoid damaging large blood vessels and bile ducts. Liver samples (1.5–2.0 cm in length) were collected. If the animals died during the experiments, their liver tissues would be taken for pathological examinations immediately. At the sixteenth week, surviving cynomolgus monkeys were sacrificed by air embolism (air injection through ear veins), and liver samples (1.5 cm × 1.0 cm × 0.3 cm) were collected for pathological examinations.

1.4. Pathological examinations

Liver tissues were fixed with 10% formaldehyde and paraffin embedded and sections were cut for hematoxylin-eosin (H&E) staining and Masson's trichrome stain. Dynamic pathological changes of liver fibrogenesis were monitored in the course of the entire experiments. According to the guideline of the Xi'an Conference (2000) of the Chinese Society of Hepatology, Chinese Medical Association (Chinese Medicine Association, 2001), liver fibrosis is classified into five stages based on hyperplasia of the liver fibrous tissues, S0–S4, where S1 and S2 are mild liver fibrosis and S3 and S4 are severe liver fibrosis (S4 is the early cirrhosis). Degrees of inflammation in liver tissues were rated from G1 to G4 (G4 being the highest degree of inflammation).

1.5. Statistical analysis

All the serum samples were collected from animals at different liver fibrosis stages. SPSS Software version 18.0 was used to analyze the abovementioned serum markers. Differences between parameters were analyzed by ANOVA. Differences between groups were evaluated by SNK q tests. P value <0.05 indicates statistical significance.

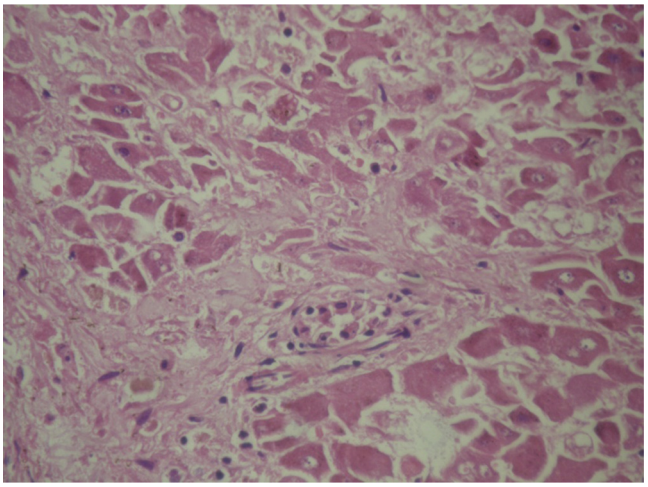

Fig. 2. Pathological examination of the liver tissues (H&E stain, 400×). Large area of necrotic hepatocytes and a low degree of fibrogenesis are shown.

2. Results

2.1. Survival of cynomolgus monkeys during experiments

Five of the 20 cynomolgus monkeys died during the experimental process, two of which died one day after the first CCl_4 administration, one after the second CCl_4 administration and the other two one day after liver biopsy at the 4th and 8th weeks, respectively. The remaining animals were stable. Pathological examinations demonstrated that the three animals that died after the first and second drug administrations showed no liver fibrosis with only large areas of swollen and necrotic hepatocytes (Fig. 1), whereas the two that died the next day after liver biopsy had large areas of liver necrosis and mild fibrosis (Fig. 2).

2.2. Progression of liver fibrosis

Pathological examinations demonstrated that liver fibrosis progressed gradually with time (Figs. 3–6) and liver fibrosis models

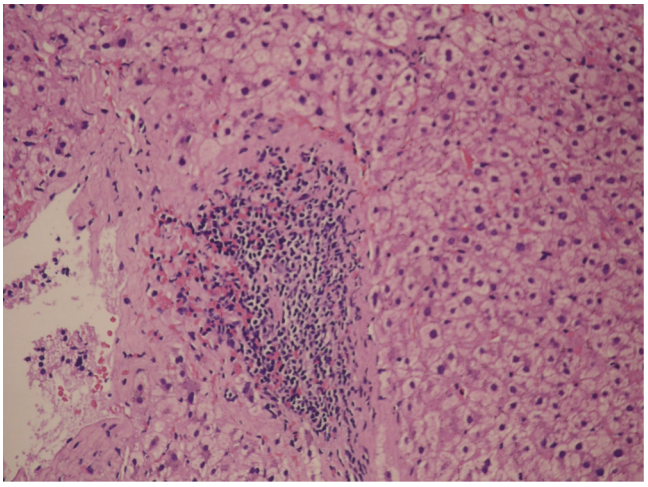

Fig. 3. Liver fibrosis S1 stage (H&E stain, 200×): enlarged portal regions, intact hepatic lobules surrounded by localized fibrous connective tissue, but no fibrous septa were observed.