

## Activation of Stellate Cells Before Induction of Hepatic Fibrosis – Precise Timing in Choline-deficient Diet-fed Rat Model

Kazuhiko Besshi\*<sup>1</sup>, Mutsunori Fujiwara<sup>2</sup>, Haruki Senoo<sup>3</sup>, Yasufumi Kondou<sup>1</sup>, Yoshitaka Ohsugi<sup>1</sup>, Taiji Hayashi<sup>1</sup> and Kozo Ishidate<sup>4</sup>

Address: <sup>1</sup>Drug Safety Research Laboratory, Taiho Pharmaceutical Co., Ltd., 224-2 Ebisuno, Hiraishi, Kawauchi-cho, Tokushima, Japan, <sup>2</sup>Division of Pathology, Japanese Red Cross Medical Center, Japan, <sup>3</sup>Department of Anatomy, Akita University School of Medicine, Japan and <sup>4</sup>Department of Molecular Cell Biology, Medical Research Institute, Tokyo Medical and Dental University, Japan

Email: Kazuhiko Besshi\* - kazu-besshi@taiho.co.jp; Mutsunori Fujiwara - m-fujiwara@mbg.sphere.ne.jp; Haruki Senoo - senoo@ipc.akita-u.ac.jp; Yasufumi Kondou - kazu-besshi@taiho.co.jp; Yoshitaka Ohsugi - kazu-besshi@taiho.co.jp; Taiji Hayashi - kazu-besshi@taiho.co.jp; Kozo Ishidate - kazu-besshi@taiho.co.jp

\* Corresponding author

from 11th International Symposium on the Cells of the Hepatic Sinusoid and their Relation to Other Cells  
Tucson, Arizona, USA, 25–29 August, 2002

Published: 14 January 2004

*Comparative Hepatology* 2004, **3**(Suppl 1):S14

This article is available from: <http://www.comparative-hepatology.com/content/3/S1/S14>

### Introduction

Quiescent hepatic stellate cells (HSCs) store vitamin A as lipid droplets in the cytoplasm. The activated HSCs by several stimuli have functions similar to that of myofibroblasts and play key roles in hepatic fibrosis [1-3]. However, precise timing between activation of the HSC and induction of hepatic fibrosis is still unknown. Choline-deficient (CD) diet induces fatty liver and subsequently hepatic fibrosis in rats. We investigated the changes of HSCs in the progress of hepatic fibrosis induced by CD diet in rats, and analyzed the time course from the activation of the HSCs to the induction of hepatic fibrosis.

### Methods

Male Sprague-Dawley rats (Charles River Japan Inc.) were divided into two groups fed CD diet (DYET#118753; Dyets Inc.) for 8 weeks or standard laboratory diet (CE-2; CLEA Japan Inc.) as control. Food and water were available *ad libitum* throughout the study period. The rats were 6 weeks of age and weighing 150 to 170 g at start of CD diet administration. Four rats of each group were sacrificed under anesthesia at 2, 4, 6 and 8 weeks, respectively, after the beginning of CD diet administration. In addition, 4 rats as pretreatment-animal were sacrificed before CD diet administration.

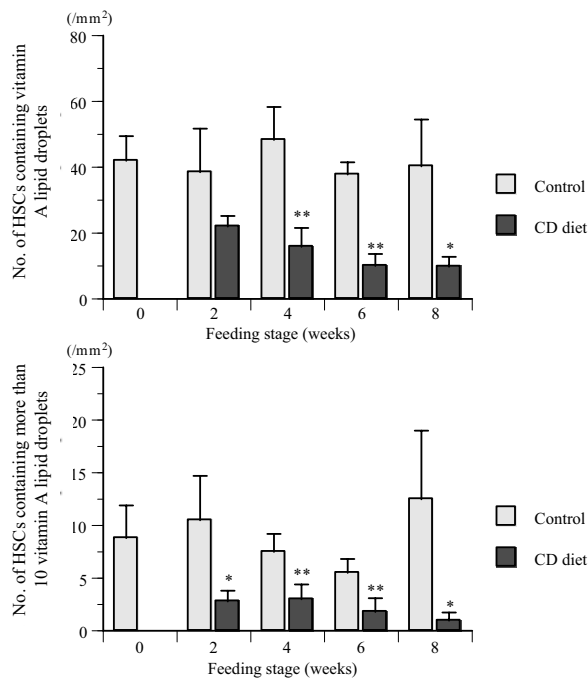
Liver tissues, obtained from sacrificed rats, were fixed 2.5% glutaraldehyde and 1% osmium tetroxide and

embedded in epoxy resin for electron microscopy. We counted microscopically the number of HSCs containing vitamin A lipid droplets in methylene blue-stained thick sections from epoxy-embedded tissue blocks. HSCs containing more than 10 vitamin A lipid droplets were defined as vitamin A-rich HSCs. Tissues for light microscopy were fixed in buffered 10% formalin and embedded in paraffin, and sections were stained with hematoxylin-eosin or silver impregnation. We observed development of fibrosis using these specimens. For detection of activated HSCs, sections for light microscopy were immunohisto-chemically stained with anti alpha-smooth muscle actin antibody (alpha-SMA monoclonal antibody, Dako Japan, Inc.). We counted microscopically the number of alpha-SMA positive HSCs.

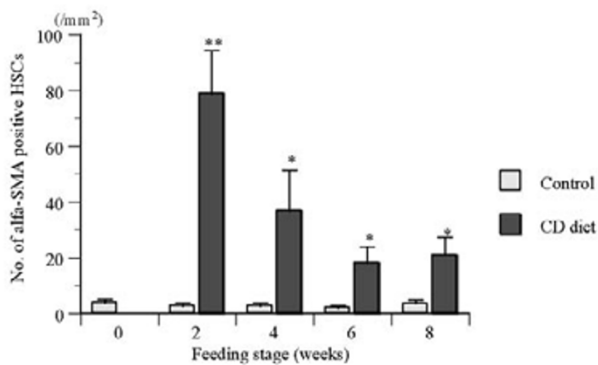
The number of HSCs (/mm<sup>2</sup>) were shown as the mean ± standard deviation. The data in CD diet group were compared with that in control group by t-test.

### Results

The number of HSCs (/mm<sup>2</sup>) containing vitamin A lipid droplets are shown in figure 1. The number of HSCs containing vitamin A lipid droplets in CD diet group after 2 weeks-feeding was fewer than that in control group, however, there was no statistically significant difference between two groups. Thereafter, the number of HSCs in CD diet decreased further, and were fewer with significant



**Figure 1**  
Number of hepatic stellate cells (HSCs) containing vitamin A lipid droplets in male rats fed choline-deficient (CD) diet for 2, 4, 6, and 8 weeks. Significant difference from control group (fed standard laboratory diet): \*)  $p < 0.05$ , \*\*)  $p < 0.01$



**Figure 2**  
Number of alpha-smooth muscle actin (alpha-SMA) positive hepatic stellate cells (HSCs) in male rats fed choline-deficient (CD) diet for 2, 4, 6, and 8 weeks. Significant difference from control group (fed standard laboratory diet): \*)  $p < 0.05$ , \*\*)  $p < 0.01$

difference ( $p < 0.01$  or  $0.05$ ) at 4, 6 and 8 weeks after the beginning of CD diet administration. The number of vitamin A-rich HSCs, containing more than 10 vitamin A lipid droplets, in CD diet were fewer with significant difference ( $p < 0.01$  or  $0.05$ ) after 2, 4, 6, and 8 weeks-feeding.

The number of alpha-SMA positive HSCs (/mm<sup>2</sup>) are shown in figure 2. The number of alpha-SMA positive HSCs in CD diet group were larger with significant difference ( $p < 0.01$  or  $0.05$ ) than that in control group after 2, 4, 6, and 8 weeks-feeding.

In histopathological examination using specimens stained with hematoxylin-eosin or silver impregnation, CD diet group showed no fibrosis after 2 weeks-feeding, but showed slight fibrosis in the pericentral and periportal regions after 4 weeks-feeding. Fibrosis in CD diet group was progressive, and pseudolobules were formed after 8 weeks-feeding. In addition, CD diet group showed cytoplasmic vacuolization of hepatocytes (fatty change) from 2 weeks to 8 weeks-feeding.

## Discussion

Quiescent HSCs store vitamin A as lipid droplets in the cytoplasm. The HSCs are transformed to myofibroblast-like cells when showing a decrease in number of vitamin A lipid droplets, activated by several stimuli and express alpha-SMA [1-5].

In our present study, the number of HSCs containing vitamin A lipid droplets decreased, and alpha-SMA positive HSCs increased after 2 weeks-feeding CD diet. However, CD diet group showed histologically no hepatic fibrosis after 2 weeks-feeding. Thereafter, slight fibrosis in the pericentral and periportal regions was observed after 4 weeks-feeding. Fibrosis was progressive, and pseudolobules were formed after 8 weeks-feeding CD diet.

It is reported that hepatic vitamin A decreases in hepatic fibrosis and cirrhosis [5,6]. In rat and human, approximately 90% of total body vitamin A is stored in the liver [7]. HSCs take up retinol-retinol binding protein as vitamin A from blood stream [8], and store approximately 75% of the total hepatic vitamin A [7]. It was unclear whether decrease of vitamin A in HSCs in fibrosis/cirrhosis precedes the induction of fibrosis/cirrhosis or is the consequence of fibrosis/cirrhosis. Our study is the first report demonstrating that transformation of HSCs from quiescent to activated phenotype precedes 2 weeks before induction of fibrosis in the CD diet-fed rat model, and suggests that this transformation of HSCs is a cause of hepatic fibrosis.

## References

1. Wake K: **Perisinusoidal stellate cells (fat-storing cells, interstitial cells, lipocytes), their related structure in and around the liver sinusoids, and vitamin A-storing cells in extrahepatic organs.** *Int Rev Cytol* 1980, **66**:303-353.
2. Blomhoff R, Wake K: **Perisinusoidal stellate cells of the liver: important roles in retinol metabolism and fibrosis.** *FASEB J* 1991, **5**:271-277.
3. Friedman SL: **The cellular basis of hepatic fibrosis, mechanisms and treatment strategies.** *New England J Med* 1993, **328**:1828-1835.
4. Friedman SL: **Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury.** *J Biol Chem* 2000, **275**:2247-2250.
5. Senoo H, Wake K: **Suppression of experimental hepatic fibrosis by administration of vitamin A.** *Lab Invest* 1985, **52**:182-194.
6. Lieber CS, Garro A, Leo MA, Mak KM, Worner T: **Alcohol and cancer.** *Hepatology* 1986, **6**:1005-1019.
7. Hendriks HFJ, Verhoofstad WAMM, Brouwer A, De Leeuw AM, Knook DL: **Perisinusoidal fat-storing cells are the main vitamin A storage sites in rat liver.** *Exp Cell Res* 1985, **160**:138-149.
8. Blomhoff R, Norum KR, Berg T: **Hepatic uptake of [<sup>3</sup>H] retinol bound to the serum retinol binding protein involves both parenchymal and perisinusoidal stellate cells.** *J Biol Chem* 1985, **260**:13571-13575.

Publish with **BioMed Central** and every scientist can read your work free of charge

*"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."*

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:  
[http://www.biomedcentral.com/info/publishing\\_adv.asp](http://www.biomedcentral.com/info/publishing_adv.asp)

