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Letter to the Editor Open Access

# An SHR/NDmcr-cp Rat Model of Non-alcoholic Steatohepatitis with Advanced Fibrosis Induced by a High-fat, High-cholesterol Diet

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## Letter to the Editor

Non-alcoholic steatohepatisis (NASH) is part of the spectrum of non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of metabolic syndrome including obesity [1,2]. In NASH, not only hepatic fat accumulation, but also various degrees of inflammation and fibrosis are present. NASH may progress to liver cirrhosis, but the causes of progression to fibrosing steatohepatitis remain unclear [1-3]. Although rodent models of NASH/NAFLD develop steatosis within the short term, fibrosis of the liver rarely develops and requires a long period to develop [1,3-6]. We have previously reported that an animal model of metabolic syndrome, the spontaneously-hypertensive/NIH-corpulent (SHR/NDmcr-cp) rat, could offer a model of NASH when fed normal diet (AIN-93G; 64.0% from carbohydrates; 16.0% from fat; and 20% from protein) for 15 weeks, but showed no histological signs of hepatic fibrosis (stage 0 in all 5 rats) according to the NASH Clinical Research Network Scoring System [7,8]. Feeding these rats a normal diet (AIN-93G) for 23 weeks led to only slight fibrosis (stage 0 in 3 rats and stage 1A in 2 rats) [7,9]. Recent reports suggest that dietary cholesterol is a critical factor in the development of experimental steatohepatitis in animal models. Savard et al. reported that dietary fat and dietary cholesterol interact synergistically to induce the metabolic and hepatic features of NASH, while neither factor alone is sufficient to cause NASH in male C57BL/6J littermate mice [3].

In this study, 8-week-old male SHR/NDmcr-cp rats (n=5) were purchased from Nippon SLC (Hamamatsu, Japan). After 1 week of acclimation, rats were given a high-fat, high-cholesterol (HFC) diet containing 5% cholesterol and 2% cholic acid. This HFC diet comprised the following energy sources: 42.9% from carbohydrates; 28.6% from fat; and 14.7% from protein (overall calories: 487.8 kcal/100 g). All rats were housed individually in a temperature- and humidity-controlled environment with a 12:12-h light-dark cycle. Rats did not have the opportunity to move, and the group was homogeneous. Rats were fed for 9 weeks, then killed under anesthesia following overnight fasting. Blood samples were taken from the inferior vena cava for the measurement of serological parameters. The livers were removed, fixed in 10% neutral-buffered formalin, processed, and embedded in paraffin for hematoxylin-eosin and Azan staining for histopathological examinations. All procedures were performed in accordance with the Guidelines for Animal Experimentation and approved by the Animal Usage Committee of the University of Nagasaki.

At 18 weeks old, SHR/NDmcr-cp rats showed obesity (647 ± 20 g, mean ± standard error). Serum total cholesterol levels were considerably elevated. Histopathological findings were scored using the NASH Clinical Research Network Scoring System based on four semi-quantitative factors: steatosis (0-3); lobular inflammation (0-3); hepatocyte ballooning (0-2); and fibrosis (0-4). NAFLD activity score (NAS) was defined as the unweighted sum of the scores for steatosis, lobular inflammation, and hepatocyte ballooning (range, 0-8). NAS scores ≥ 5 are diagnostic of steatohepatitis [7]. Severe micro- or macrovesicular fatty changes were found in the liver of each rat. Obvious lobular inflammation and hepatocyte ballooning were also seen. Furthermore, fibrosis (stage 2-4) was observed in all rats. All rats were diagnosed with steatohepatitis according to NAS score (score 7-8) (Table 1). SHR/NDmcr-cp rats fed a high-fat diet containing 5% cholesterol thus develop steatohepatitis with significant fibrosis (cirrhosis) within a shorter period than reported in previous studies [3,6].

| Rat | Serological data | | | | | | | | Histopathological findings* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glucose (mg/dL) | Insulin (ng/mL) | Total cholesterol (mg/dL) | Triglyceride (mg/dL) | Leptin (ng/mL) | Adiponectin (μg/mL) | AST (IU/L) | ALT (IU/L) | Steatosis | Lobular inflammation | Hepatocyte ballooning | NAS** | Fibrosis |
| 1 | 146 | 20 | 781 | 271 | 141 | 7.2 | 787 | 609 | 3 | 3 | 1 | 7 | 2 |
| 2 | 103 | 11 | 976 | 494 | 135 | 3.5 | 1,459 | 1000 | 3 | 3 | 2 | 8 | 2-3 |
| 3 | 56 | 1 | 1,055 | 371 | 44 | 1.1 | 1,546 | 578 | 3 | 3 | 2 | 8 | 3-4 |
| 4 | 192 | 1 | 979 | 2,052 | 16 | 3.6 | 565 | 210 | 3 | 2 | 2 | 7 | 4 |
| 5 | 84 | 8 | 840 | 397 | 157 | 0.8 | 400 | 287 | 3 | 3 | 2 | 8 | 4 |

**Table 1:** Serological and histopathological evaluation of the liver in SHR/NDmcr-cp rats (18 weeks of age).

*Histopathological findings were scored using the non-alcoholic steatohepatitis (NASH) Clinical Research Network Scoring System [9]. **Non-alcoholic fatty liver disease activity score (NAS) is defined as the unweighted sum of scores for steatosis (0-3), lobular inflammation (0-2), and hepatocyte ballooning (0-4), for a final score ranging from 0 to 8. NAS scores ≤2 are considered not diagnostic of steatohepatitis; scores ≥5 are diagnostic of steatohepatitis [9]. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

There are some limitations to the present study. First, there were no control SHR/NDmcr-cp rats fed a normal or low-fat diet. Therefore, it cannot be determined whether the results are significant. Second, data presented were from a small sample size (n=5). Third, acceleration of fibrosis by dietary cholic acid (2%) cannot be ruled out in our model. However, it is noted that SHR/NDmcr-cp rats fed a high-fat diet containing 5% cholesterol developed steatohepatitis with significant fibrosis (cirrhosis) within a shorter period than reported in previous studies [10]. Moreover, the progression of NASH-related cirrhosis in our NASH model reflects the human etiology that is influenced by dietary factors including cholesterol [11], and therefore, the model could be useful for elucidating how NASH progresses towards fibrosis/cirrhosis.

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