Maraviroc improves hepatic triglyceride content but not inflammation in a murine nonalcoholic fatty liver disease model induced by a chronic exposure to high-fat diet

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Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the general population. Its severity ranges from simple steatosis to cirrhosis. C-C chemokine ligand type 5 or RANTES plays an important role in the progression of hepatic inflammation and fibrosis. Our objective was to examine the preventive and therapeutic effects of maraviroc (MVC), a C-C chemokine receptor 5 antagonist, on liver pathology in an NAFLD mouse model. A total of 60 male C57BL/6 mice were randomly assigned to 1 of 4 groups: (1) high-fat diet (HFD) group or control group, (2) preventive group (HFD group plus MVC in drinking water since the beginning of the study), (3) early-therapeutic group (HFD group plus MVC in drinking starting at week 24 of the study), and (4) late-therapeutic group (HFD group plus MVC in drinking water starting at week 36 of the study). All mice were sacrificed at week 48. The hepatic triglyceride concentration in the HFD group was significantly higher than that in the groups treated with MVC at any time. Gene expression associated with lipogenesis (diacylglycerol acyltransferase 2 and proliferator-activated receptor-γ), insulin resistance (insulin receptor substrate-2), and β-oxidation (carnitine palmitoyltransferase 1A and acyl-CoA oxidase) was significantly reduced in all the groups treated with MVC. In summary, the beneficial effect of MVC on hepatic steatosis is maintained throughout the study. (Translational Research 2018;■■:■■–■■)

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathologic syndrome characterized by triglyceride (TGD) accumulation (hepatosteatosis) in the absence of chronic viral hepatitis infection or alcohol consumption.[1] NAFLD includes a wide spectrum of liver diseases with very different natural course and prognosis, including patients with isolated hepatic steatosis and others with nonalcoholic steatohepatitis (NASH), a more aggressive disease characterized by steatosis and hepatocellular injury with or without fibrosis.[2] Cases of NASH can evolve into cirrhosis and end-stage liver disease, including hepatocellular carcinoma (HCC).[1-4]

Currently, NAFLD is considered the most common cause of liver disease worldwide,[2] and its prevalence is increasing because of the rising incidence of diabetes and obesity.[5] In fact, NAFLD is considered the hepatic manifestation of metabolic syndrome.[6]

To date, there are significant gaps in the knowledge about NAFLD pathogenesis, prognosis, prevention, and treatment. For this reason, a better understanding of NAFLD disease is crucial. At the moment, although a perfect animal model that mimics all the histologic stages

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AT A GLANCE COMMENTARY

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Background

Nonalcoholic fatty liver disease (NAFLD) is considered the most common cause of liver disease worldwide with increasing prevalence. Because C-C chemokine receptor 5 (CCR5) expression is elevated in the liver of obese humans and mice, CCR5 blockade could be an interesting option against NAFLD. Our aim was to evaluate the preventive and therapeutic effects of maraviroc, a CCR5 antagonist, in an experimental mouse model of NAFLD.

Translational Significance

In this study, groups treated with maraviroc had (or showed) significantly lower liver triglyceride content and downregulated genes associated with lipogenesis and β-oxidation. The proposed strategy provides a protective effect on liver steatosis.

and manifestations of the human NAFLD does not exist, mice are commonly employed as animal models because they are able to reproduce many of the characteristics of human NAFLD. To date, several mouse models of NAFLD have been generated using different diet-based methods[7-10] or using genetic models.[11,12] However, the heterogeneity of the diets used makes it difficult to compare studies from different research groups.[13]

On a molecular level, chemokines play a major role in the health of the immune system. The C-C chemokine receptor 5 (CCR5)[14] and its ligand, C-C chemokine ligand type 5 (CCL5 or RANTES), are implicated in liver inflammation and fibrosis.[15-18] In addition, gene targeting or the use of a potent antagonist for the murine CCR5 receptor results in a significant reduction of liver fibrosis[16,17,19-21] and HCC.[20,22] Because CCR5 expression is elevated in the liver in obese humans and mice,[23,24] a CCR5 antagonist could be an interesting option against NAFLD. Several small-molecule antagonists of CCR5 have been evaluated as therapeutic options for the treatment of HIV-infected patients; however, only one is currently approved for clinical use, namely, maraviroc (MVC).[25] Previously, we were able to demonstrate that MVC was able to prevent the development of HCC[20] and to ameliorate the development of hepatic steatosis[26] in mouse models. In a similar way, cenicriviroc, a dual CCR5-CCR2 antagonist (cenicriviroc), has shown antifibrotic effects in animal models of liver fibrosis[27] and NAFLD subjects with liver fibrosis.[28,29]

In the current study, our aim was to evaluate the preventive and therapeutic effects of MVC in an experimental mouse model of NAFLD induced by a high-fat diet (HFD).

MATERIALS AND METHODS

Ethics statement. All procedures were carried out in accordance with the European Communities Council Directive (86/609/CEE) on animal experiments and with approval from the ethical committee on animal welfare of our institution (Comité Etico de Experimentación Animal del Centro de Investigación Biomédica de La Rioja [CEEA-CIBIR]).

Animals and animal model. A total of 60 male C57BL/6 mice were purchased from Charles River (Barcelona, Spain). All animals had free access to food and drink during the study. When the animals were approximately 5 weeks old, they were randomly assigned (n = 15) to 1 of 4 groups and fed for 48 weeks. The HFD group received an HFD (Research Diets D12492; Research Diets, Inc, N Jersey) and tap water. The preventive group (PG) received the same diet as the HFD group and received 300 mg/L MVC (Pfizer, New York, N York) in their drinking water from the beginning of the study. Mouse-equivalent drug doses were calculated using an interspecies allometric scaling factor to arrive at a dose for mice that is equivalent to a human dose of 300 mg/day.[20,26,30] The early-therapeutic group (ETG) received the same diet as the HFD group and received MVC in the drinking water at the same concentration as the PG starting from week 24 of the experiment. Finally, the late-therapeutic group (LTG) received the same diet as the HFD group and received MVC in the drinking water at the same concentration as the PG starting from week 36 of the experiment. The rationale for the initiation of the early-therapeutic model was based on the results previously obtained with this model.[26]

The mice were observed daily, and all the observations were recorded. In addition, the animals were weighted once a week. All the animals were sacrificed at week 48. At that time, blood samples were collected under anesthesia after a 4-hour fasting period. The internal organs were examined macroscopically, photographed, and weighted. Some tissues were fixed in buffered formalin for histologic analysis, and the rest were snap-frozen in liquid nitrogen for biochemical and molecular analyses.

Blood sampling and analysis. Plasma levels of aspartate aminotransferase, alanine aminotransferase (ALT), glucose, TGD, and total cholesterol (TC) were measured using an automatic biochemical analyzer (Cobas C711, Roche, Madrid, Spain). Serum high-density lipoprotein cholesterol (HDL-c) was measured using HDL-c