

Whey-hydrolyzed peptide-enriched immunomodulating diet prevents progression of liver cirrhosis in rats

Kanta Jobara

Abstract

Objective: Liver fibrosis and subsequent cirrhosis is a major cause of death worldwide, but few effective anti-fibrotic therapies are reported. Whey-hydrolyzed peptide (WHP), a major peptide component of bovine milk, exerts anti-inflammatory effects in experimental models. A WHP-enriched diet is widely used for immunomodulating diets (IMD) in clinical fields. However, the impact of WHP on liver fibrosis remains unknown. Here, we investigated the anti-fibrotic effects of WHP in a rat cirrhosis model.

Methods: Progressive liver fibrosis was induced by repeated intraperitoneal administration of dimethylnitrosamine (DMN) for 3 wk. Rats were fed either a WHP-enriched IMD (WHP group) or a control enteral diet (Control group). The degree of liver fibrosis was compared between groups. Hepatocyte-protective effects were examined using hepatocytes isolated from rats fed a WHP diet. Reactive oxygen species (ROS) and glutathione (GSH) in liver tissue were investigated in the DMN cirrhosis model.

Results: Macroscopic and microscopic progression of liver fibrosis was remarkably suppressed in the WHP group. Elevated serum levels of liver enzymes and hyaluronic

acid, and liver tissue hydroxyproline content were significantly attenuated in the WHP group. Necrotic hepatocyte rates with DMN challenge, isolated from rats fed a WHP-enriched IMD, were significantly lower. In the DMN cirrhosis model, ROS were significantly lower, and GSH was significantly higher in whole liver tissue in the WHP group.

Conclusion: A WHP-enriched IMD effectively prevented progression of DMN-induced liver fibrosis in rats via a direct hepatocyte-protective effect and an antioxidant effect through GSH synthesis.

References

1. Stauffer JK, Scarzello AJ, Jiang Q, Wiltout RH. Chronic inflammation, immune escape, and oncogenesis in the liver: a unique neighborhood for novel intersections. *Hepatology*. 2012;56(4):1567-74.
2. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. *Hepatology*. 1994;20:15-20.
3. Zhang DY, Friedman SL. Fibrosis-dependent mechanisms of hepatocarcinogenesis. *Hepatology*. 2012;56:769-75.
4. Pawlotsky JM. Therapy of hepatitis C: from empiricism to eradication. *Hepatology*. 2006;43:S207-20.
5. Mendes FD, Kim WR, Pedersen R, Therneau T, Lindor KD. Mortality attributable to cholestatic liver disease in the United States. *Hepatology*. 2008;47:1241-7.
6. Tsochatzis EA, Bosch J, Burroughs AK. New therapeutic paradigm for patients with cirrhosis. *Hepatology*. 2012;56:1983-92.

7. Friedman SL. Mechanisms of hepatic fibrogenesis. *Gastroenterology*. 2008;134:1655-69.
8. Gressner AM, Weiskirchen R. Modern pathogenetic concepts of liver fibrosis suggest stellate cells and TGF-beta as major players and therapeutic targets. *J Cell Mol Med*. 2006;10:76-99.
9. Parola M, Robino G. Oxidative stress-related molecules and liver fibrosis. *J Hepatol*. 2001;35:297-306.
10. Fried A, Manske SL, Eller LK, Lorincz C, Reimer RA, Zernicke RF. Skim milk powder enhances trabecular bone architecture compared with casein or whey in diet-induced obese rats. *Nutrition* 2012;28:331-5.
11. Marshall K. Therapeutic applications of whey protein. *Altern Med Rev*. 2004;9:136-56.
12. Yamaguchi M, Matsuura M, Kobayashi K, Sasaki H, Yajima T, Kuwata T. Lactoferrin protects against development of hepatitis caused by sensitization of Kupffer cells by lipopolysaccharide. *Clin Diagn Lab Immunol*. 2001;8:1234-9.
13. Kume H, Okazaki K, Sasaki H. Hepatoprotective effects of whey protein on D-galactosamine-induced hepatitis and liver fibrosis in rats. *Biosci Biotechnol Biochem*. 2006;70:1281-5.
14. Nakamura K, Ogawa S, Dairiki K, Fukatsu K, Sasaki H, Kaneko T, et al. A new immune-modulating diet enriched with whey-hydrolyzed peptide, fermented milk, and isomaltulose attenuates gut ischemia-reperfusion injury in mice. *Clin Nutr*. 2011;30:513-6.
15. Takayanagi T, Sasaki H, Kawashima A, Mizuochi Y, Hirate H, Sugiura T, et al. A

new enteral diet, MHN-02, which contains abundant antioxidants and whey peptide, protects against carbon tetrachloride-induced hepatitis. JPEN J Parenter Enteral Nutr. 2011;35:516-22.

16. Perrone F, da-Silva-Filho AC, Adorno IF, Anabuki NT, Leal FS, Colombo T, et al. Effects of preoperative feeding with a whey protein plus carbohydrate drink on the acute phase response and insulin resistance. A randomized trial. Nutr J. 2011;10:66.

17. Marimuthu K, Varadhan KK, Ljungqvist O, Lobo DN. A meta-analysis of the effect of combinations of immune modulating nutrients on outcome in patients undergoing major open gastrointestinal surgery. Ann Surg. 2012;255:1060-8.

18. Kaido T, Ogura Y, Ogawa K, Hata K, Yoshizawa A, Yagi S, et al. Effects of post-transplant enteral nutrition with an immunomodulating diet containing hydrolyzed whey peptide after liver transplantation. World J Surg. 2012 ;36:1666-71.

19. Jenkins SA, Grandison A, Baxter JN, Day DW, Taylor I, Shields R. A dimethylnitrosamine-induced model of cirrhosis and portal hypertension in the rat. J Hepatol. 1985;1:489-99.

20. Yoshikawa A, Kaido T, Seto S, Katsuura Y, Imamura M. Activated protein C prevents multiple organ injury following extensive hepatectomy in cirrhotic rats. J Hepatol. 2000;33:953-60.

21. Friedman SL. Evaluation of fibrosis and hepatitis C. Am J Med. 1999;107:27-30.

22. Mencin A, Seki E, Osawa Y, Kodama Y, De Minicis S, Knowles M, et al. Alpha-1 antitrypsin Z protein (PiZ) increases hepatic fibrosis in a murine model of cholestasis. Hepatology. 2007;46:1443-52.

23. Kim DH, Yang KH, Johnson KW, Holsapple MP. Role of the transfer of metabolites from hepatocytes to splenocytes in the suppression of in vitro antibody

response by dimethylnitrosamine. *Biochem Pharmacol.* 1988;37:2765-71.

24. Tamaki N, Hatano E, Taura K, Tada M, Kodama Y, Nitta T, et al. CHOP deficiency attenuates cholestasis-induced liver fibrosis by reduction of hepatocyte injury. *Am J Physiol Gastrointest Liver Physiol.* 2008;294:498-505.

25. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods.* 2001;25:402-8.

26. Toda K, Kumagai N, Kaneko F, Tsunematsu S, Tsuchimoto K, Saito H, et al. Pentoxifylline prevents pig serum-induced rat liver fibrosis by inhibiting interleukin-6 production. *J Gastroenterol Hepatol.* 2009;24:860-5.

27. Andrade Wde C, Silva LF, Coelho MC, Tannuri AC, Alves VA, Tannuri U. Effects of the administration of pentoxifylline and prednisolone on the evolution of portal fibrogenesis secondary to biliary obstruction in growing animals: immunohistochemical analysis of the expression of TGF- β and VEGF. *Clinics (Sao Paulo).* 2012;67:1455-61.

28. Costelli P, Bossola M, Muscaritoli M, Grieco G, Bonelli G, Bellantone R, et al. Anticytokine treatment prevents the increase in the activity of ATP-ubiquitin- and Ca²⁺-dependent proteolytic systems in the muscle of tumour-bearing rats. *Cytokine.* 2002;19:1-5.

29. Kume H, Okazaki K, Yamaji T, Sasaki H. A newly designed enteral formula containing whey peptides and fermented milk product protects mice against concanavalin A-induced hepatitis by suppressing overproduction of inflammatory cytokines. *Clin Nutr.* 2012;31:283-9.

30. Sugawara K, Takahashi H, Kashiwagura T, Yamada K, Yanagida S, Homma M, et al. Effect of anti-inflammatory supplementation with whey peptide and exercise therapy in patients with COPD. *Respir Med.* 2012;106:1526-34.

31. Terblanche J, Hickman R. Animal models of fulminant hepatic failure. *Dig Dis Sci*. 1991;36:770-4.
32. Leist M, Gantner F, Bohlinger I, Tiegs G, Germann PG, Wendel A. Tumor necrosis factor-induced hepatocyte apoptosis precedes liver failure in experimental murine shock models. *Am J Pathol*. 1995;146:1220-34.
33. Takehara T, Tatsumi T, Suzuki T, Rucker EB, 3rd, Hennighausen L, Jinushi M, et al. Hepatocyte-specific disruption of Bcl-xL leads to continuous hepatocyte apoptosis and liver fibrotic responses. *Gastroenterology*. 2004;127:1189-97.
34. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of hepatotoxicity. *Toxicol Sci*. 2002;65:166-76.
35. Haggerty HG, Holsapple MP. Role of metabolism in dimethylnitrosamine-induced immunosuppression: a review. *Toxicology*. 1990;63:1-23.
36. Lin J, Zhao J, Li T, Zhou J, Hu J, Hong Z. Hepatoprotection in a rat model of acute liver damage through inhibition of CYP2E1 activity by total alkaloids extracted from *Rubus alceifolius* Poir. *Int J Toxicol*. 2011;30:237-43.
37. Meng B, Gao W, Wei J, Yang J, Wu J, Pu L, et al. Quercetin reduces serum homocysteine level in rats fed a methionine-enriched diet. *Nutrition* 2013;29:661-6.