Inhibition of matrix metalloproteinases increases PPAR-α and IL-6 and prevents dietary-induced hepatic steatosis and injury in a murine model

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Alwayn, Ian P. J., Charlotte Andersson, Sang Lee, Danielle A. Arsenault, Bruce R. Bistrian, Kathleen M. Gura, Vanja Nose, Blanca Zauscher, Marsha Moses, and Mark Puder. Inhibition of matrix metalloproteinases increases PPAR-α and IL-6 and prevents dietary-induced hepatic steatosis and injury in a murine model. Am J Physiol Gastrointest Liver Physiol 291: G1011–G1019, 2006. First published July 13, 2006; doi:10.1152/ajpgi.00047.2006.—Steatosis is a prominent feature of nonalcoholic fatty liver disease and a potential promoter of inflammation. Injury leading to cirrhosis is partly mediated by dysregulation of matrix protein turnover. Matrix metalloproteinase (MMP) inhibitors protect mice from lethal TNF-α induced liver injury. We hypothesized that Marimastat, a broad-spectrum MMP and TNF-α converting enzyme (TACE) inhibitor, might modulate this injury through interruption of inflammatory pathways. Triglyceride and phospholipid levels (liver, serum) and fatty acid profiles were used to assess essential fatty acid status and de novo lipogenesis as mechanisms for hepatic steatosis. Mice receiving a fat-free, high-carbohydrate diet (HCD) for 19 days developed severe fatty liver infiltration, demonstrated by histology, magnetic resonance spectroscopy, and elevated liver function tests. Animals receiving HCD plus Marimastat (HCD+MAR) were comparable to control animals. Increased tissue levels of peroxisome proliferator activated receptor-α (PPAR-α), higher levels of serum IL-6, and decreased levels of serum TNF-α receptor II were also seen in the HCD+MAR group compared with HCD-only. In addition, there was increased phosphorylation, and likely activation, of PPAR-α in the HCD+MAR group. PPAR-α is a transcription factor involved in β-oxidation of fatty acids, and IL-6 is a hepatoprotective cytokine. Liver triglyceride levels were higher and serum triglyceride and phospholipid levels lower with HCD-only but improved with Marimastat treatment. HCD-only and HCD+MAR groups were essential fatty acid deficient and had elevated rates of de novo lipogenesis. We therefore conclude that Marimastat reduces liver triglyceride accumulation by increasing fat oxidation and/or liver clearance of triglycerides. This may be related to increased expression and activation of PPAR-α or IL-6, respectively.

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NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) is associated with other metabolic disorders such as obesity (46, 49, 60) and diabetes (13) and consists of a spectrum of pathological states ranging from the simple buildup of fat in the liver (hepatic steatosis) to nonalcoholic steatohepatitis, cirrhosis, and ultimately liver failure (2, 4, 10, 12, 58). NAFLD is being increasingly appreciated as a major cause of liver-related morbidity and mortality. A recent survey has indicated that NAFLD accounts for 80% of patients that present with elevated serum aminotransferases (11) and that up to 30% of the population in Western societies may have NAFLD (6). The mechanism for the development of hepatic steatosis can be due to increased hepatic synthesis, decreased oxidation, decreased secretion of fatty acids (triglycerides) from the liver, or some combination of these processes (16, 17). Certain transcription factors and cytokines have been shown to promote each of these actions. Peroxisome proliferator-activated receptor-α (PPAR-α) is an important transcription factor to foster the β-oxidation of fatty acids, and its agonists have been shown to inhibit tumor necrosis factor-α (TNF-α) secretion (5, 9, 20, 33, 65). Ligands for PPAR-α and negative regulators of hepatic lipogenesis are thought to be mediated by the repression of sterol regulatory element-binding protein-1c (SREBP-1c) (19). SREBP-1 is an important regulator of hepatic lipogenesis and cholesterol biosynthesis (8). Finally, IL-6 has been demonstrated to enhance triglyceride clearance from the liver (27). IL-6 alleviates hepatic steatosis and ischemia/reperfusion injury in mice with fatty liver disease (27, 57).

Matrix metalloproteinases (MMPs), a family of zinc- and calcium-dependent proteases, are capable of degrading the extracellular matrix and are involved in embryonic growth and development as well as organ remodeling and repair (42, 47). More specifically, MMP-9 has been implicated in the regulation of the proliferation and migration of hepatocytes (26, 34, 35) and may serve to recruit progenitor cells from the bone marrow to the injured liver in vivo (61). Furthermore, MMPs are induced by TNF-α as a consequence of the systemic inflammatory response and have been shown to modulate inflammation by either activation or inactivation of cytokines and growth factors (24, 41). Inhibition of MMPs protects against TNF-α-induced hepatitis and other pathologies in animal models (64). Lastly, monoclonal antibodies specific for TNF-α reduce hepatic steatosis in animal models (39).

The present study was designed to determine the specific effects of treatment with the broad-spectrum MMP and TNF-α
converting enzyme (TACE)-inhibitor Marimastat (British Bio-tech, Oxford, UK) on dietary-induced liver lipid accumulation and associated injury in mice. We demonstrate that this drug markedly limits hepatic triglyceride accumulation induced by a HCD that is fat free while increasing serum triglyceride and phospholipid levels. Associated findings of a marked increase in hepatic PPAR-α and serum IL-6 levels and unchanged hepatic SREBP-1c levels support both increased fatty acid oxidation and greater hepatic lipid secretion as the likely mechanisms for this improvement.

**MATERIALS AND METHODS**

**Animal model.** Experiments were performed on 5- to 6-wk-old C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME). The animals, in groups of five, were housed in a barrier room and were acclimated to their environment for at least 72 h before the initiation of each experiment. Animal protocols complied with the National Institutes of Health (NIH) Animal Research Advisory Committee guidelines and were approved by the Children’s Hospital Boston Animal Care and Use Committee.

Control animals (n = 5) received normal chow and water ad libitum. All animals in the experimental groups were fed a fat-free, high-carbohydrate liquid diet (HCD) ad libitum placed in one bottle per cage as previously described (3, 63). No additional sources of nutrition or hydration were provided. The HCD liquid solution contained 20% dextrose, a commercial mixture of 2% essential and nonessential amino acids (TrophAmine, B. Braun Medical, Irvine, CA), 2 ml of trace elements (Trace Elements Injection 4, USP Pediatric, American Regent, Shirley, NY), and 5 ml of multivitamins (M.V.I. Pediatric, Mayne Pharma, Paramus, NJ). Each liter of HCD contained 30 meq sodium, 20 meq potassium chloride, 15 meq calcium, 10 meq magnesium, and 10 mM phosphate. The bottles were replaced daily with fresh HCD solution. HCD animals (n = 5) received HCD only and no additional treatment for 19 days. The HCD+MAR animals (n = 15) additionally received 100 mg/kg of Marimastat in 0.45% methylcellulose (Sigma-Aldrich, St. Louis, MO) vehicle twice daily via orogastric gavage. The animals were individually weighed every third day.

**Specimen collection.** Mice were anesthetized with 300 µl of 2.5% Avertin (Tribronmoethanol, Sigma-Aldrich) by intraperitoneal injection. Approximately 400 µl of blood were collected from each mouse via retro-orbital puncture. The specimens were then placed into serum separator tubes (Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 4°C at 8,000 rpm for 10 min to separate serum. Serum was frozen at −80°C and delivered to the Clinical Laboratory at Children’s Hospital Boston for measurement of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and total and direct bilirubin levels. Furthermore, a fatty acid profile was performed on these samples and TNF-α soluble receptor II levels were determined as described.

 western blot analysis. Protein extraction was performed according to ActiveMotif nuclear extraction kit protocol (ActiveMotif, Carlsbad, CA). The lysate obtained was stored at −80°C for future analysis. Before Western blot analysis, samples were normalized for protein content by the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). A standard curve was generated by using a range of concentrations of bovine serum albumin (Sigma-Aldrich). The assay was read at 595 nm and analyzed with Softmax PRO software (Molecular Devices).

Magnetic resonance imaging. One section of liver was snap frozen and stored at −80°C for evaluation by magnetic resonance (MR) spectroscopy to determine the percentage liver fat content (2, 31). MR imaging and spectroscopy were performed on a Bruker 8.5-T magnet. The liver samples were thawed at room temperature 1 h before the MR experiments. Spin-lattice relaxation time T1 measurements were made with the saturation recovery approach using spin echo images with an echo time of 6.4 ms and 8 relaxation times ranging from 0.05 to 4,000 ms. Three 2-mm-thick slices were imaged for each sample and the saturation recovery curves were generated from signal intensities measured in identically sized regions of interest within a given slice. Care was taken to exclude macroscopic fat from the selected region of interest. Free induction decays with 1,024 time points and a 5-kHz bandwidth were also acquired from each sample by using a hard 90° radiofrequency pulse with 16 signal averages, a 10-s relaxation time, and a flip angle of 90°. Spectra were obtained following Fourier transformation and phasing, and percent fat content was determined relative to water by numerical integration of the areas under the lipid and water peaks by an independent blinded reviewer.

**Immunoprecipitation, SDS-PAGE.** Immunoprecipitation was performed according to the Protein-G immunoprecipitation kit (Sigma-Aldrich) protocol. Briefer, cell lysate with a total protein content of 1 mg was incubated with 5 µg of antibody (anti-PPAR-α antibody, MA1–822, Affinity Bioreagents, Golden, CO). The secondary antibodies used were horse-radish peroxidase (HRP)-linked donkey anti-rabbit IgG in a 1:5,000 dilution (Amersham Biosciences UK, Buckinghamshire, UK), or ECL sheep anti-mouse IgG, HRP-linked whole antibody in a 1:5,000 dilution (Amersham Biosciences UK), respectively. Equal protein loading was verified by incubating the same membrane with β-actin antibody in a 1:500 dilution (MS X Actin, Chemicon International, Temecula, CA). The probed proteins were developed by use of the Pierce enhanced chemiluminescent substrate for detection of HRP according to manufacturer’s instructions (Pierce, Rockford, IL).

**Immunoprecipitation, SDS-PAGE.** Immunoprecipitation was performed according to the Protein-G immunoprecipitation kit (Sigma-Aldrich) protocol. Briefer, cell lysate with a total protein content of 1 mg was incubated with 5 µg of antibody (anti-PPAR-α antibody, MA1–822, Affinity Bioreagents) overnight at 4°C with constant mixing. Thirty microliters of Protein-G-agarose beads were then added, and the mixture was incubated for an additional 2 h at 4°C. The beads were then washed with buffer five times, with the supernatant
being aspirated and discarded. Forty microliters of loading buffer were then added to the beads after the final wash, and the mixture was heated to 95°C for 5 min. The sample was then centrifuged and the supernatant was analyzed by SDS-PAGE as detailed above.

Statistical analysis. Comparisons of means between two experimental groups were made using two-tailed, independent t-tests. Comparisons between multiple experimental groups were performed using a one-tailed, ANOVA test. P < 0.05 was considered statistically significant. All statistical tests were performed using SigmaStat software (SPSS, Chicago, IL). Values are listed as means ± SE.

RESULTS

Marimastat ameliorates macroscopic and histological features of hepatic steatosis in C57BL/6 mice. All animals survived the protocol. Animals receiving Marimastat (HCD+MAR) developed soft stool without gross signs of loss of bowel integrity. No animals had any signs of morbidity. All experimental groups gained weight after 19 days, with an average weight gain of 10% (data not shown). There was no statistically significant difference in animal body weights between experimental groups.

Macroscopic differences in liver appearance between experimental groups were noted at the time of death. Specifically, livers from the HCD-only mice were pale yellow, suggesting fatty liver changes. There was no significant difference in liver weights between experimental groups (data not shown).

A pathologist blinded to the treatment groups conducted a histological analysis of the livers. Staining with H&E revealed occasional focal cellular infiltrates consistent with focal necrosis in the HCD+MAR group. No acute inflammatory changes were observed in any other group.

Livers from control, chow-fed mice exhibited typical hepatic architecture (Fig. 1A) and glycogen storage (Fig. 1B). As anticipated, no evidence of hepatic steatosis was noted in this group. In contrast, livers from HCD-only mice showed extensive steatosis, including both macro- and microvesicular types (Fig. 1C). Steatosis was present predominantly in the periportal and midzone hepatocytes. The two to three cell layers surrounding the central vein were preserved, ending abruptly with an immediate change to steatosis outside its perimeter. HCD-only livers also exhibited low levels of PAS-positive cells, which suggests minimal glycogen storage within hepatocytes (Fig. 1D). The few cells that were PAS positive in this group were located in the two to three cell layers surrounding the central vein that was preserved from steatosis. Livers from HCD+MAR mice exhibited fairly normal hepatic architecture, with only rare microvacuoles in the cytoplasm of midzone hepatocytes.

Fig. 1. Hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining of liver specimens from all experimental groups are represented. Control livers exhibited normal architecture and glycogen storage, whereas high-carbohydrate diet (HCD)-only livers revealed extensive microvesicular and macrovesicular steatosis and a very low level of glycogen storage. High-carbohydrate diet plus Marimastat (HCD+MAR) livers were comparable to control livers in that they showed normal hepatic architecture and minimal fat. These livers also contained a high level of glycogen storage.
hepatocytes (Fig. 1E). No definitive steatosis was identified on H&E sections in this group, and PAS staining was highly positive, granular, and widespread (Fig. 1F). Although PAS staining was diffuse, it was most prevalent around the portal and central veins and less prominent in the midzone.

Oil Red O staining revealed minimal fat in control livers (Fig. 2A). Livers from HCD-only mice exhibited diffuse presence of fat throughout the liver parenchyma (Fig. 2B), whereas livers from HCD-MAR mice showed minimal presence of fat (Fig. 2C), consistent with levels observed in the control group.

Marimastat ameliorates hepatic steatosis in C57BL/6 mice determined by MR spectroscopy. MR spectroscopy was used to quantify hepatic fat content. Baseline fat content was established by using livers from control, chow-fed mice. A close correlation was observed between the percent fat calculated from the MR spectroscopy and the degree of T1 shortening determined from the T1 relaxation curves (data not shown).

Control, chow-fed animals were found to have a liver fat content of 3.4 ± 0.6%. In contrast, HCD-only mice exhibited a significant increase in liver fat content with a value of 24.1 ± 1.7%, \( P < 0.001 \). Concomitant treatment with Marimastat (HCD+MAR) resulted in a significant decrease in liver fat content relative to HCD-only mice (8.1 ± 1.2, \( P < 0.05 \)). The HCD+MAR and control, chow-fed animals showed no significant difference in liver fat content.

Marimastat improves serum liver function tests in hepatic steatosis in C57BL/6 mice. To detect the presence of liver injury, liver function tests were conducted on all experimental groups. HCD-only animals exhibited significantly higher values than HCD+MAR animals on all tests (Fig. 3, \( P < 0.05 \)), with the exception of aspartate aminotransferase (data not shown). This suggests that the biochemical damage induced by HCD involves both liver parenchyma and bile ducts and that treatment with Marimastat can abrogate this damage. When comparing chow-fed control animals to HCD-only animals, similar results were observed, with the exception that alkaline phosphatase and direct bilirubin were not significantly different. The lowest levels for all hepatic function tests were in the HCD+MAR group.

Marimastat does not prevent essential fatty acid deficiency in C57BL/6 mice. A high-carbohydrate, low-fat diet stimulates hepatic lipogenesis (2, 31). Essential fatty acid deficiency [Mead acid-to-arachidonic acid ratio (M:A) > 0.2 in the serum lipid] fosters hepatic steatosis (31, 32, 62) principally through the process of de novo lipogenesis. In our model, maximum steatosis is achieved through the administration of a high-carbohydrate, fat-free diet. These animals are indeed essential fatty acid deficient as determined by a M:A of 0.43 ± 0.06 in the serum. In contrast, animals receiving normal Chow have a normal fatty acid status, with a M:A of 0.03 ± 0.01. The M:A of HCD+MAR, however, is 0.47 ± 0.08, confirming essential fatty acid deficiency in both HCD groups.

Marinastat decreases serum TNF-α receptor II levels. In an attempt to characterize the intensity of inflammatory events...
occurring during HCD-induced liver injury, TNF-α-soluble receptor II (TNFRII) levels were measured as described. The levels of TNFRII in HCD-only animals were significantly higher (599 ± 61) than those of HCD+MAR animals (468 ± 34, \( P < 0.05 \)), but this would represent a small difference clinically. There was no statistically significant difference when comparing circulating TNFRII levels to control animals (538 ± 48).

**Marimastat increases serum IL-6 levels.** Serum IL-6 levels were measured as described to determine whether this cytokine is involved in the clearance of fat from the liver by Marimastat. Both the HCD+MAR and the chow-fed control groups had significantly higher levels of IL-6 than the HCD-only group (\( P = 0.001, P = 0.008 \)), whereas no significant difference was observed between the HCD+MAR and chow-fed controls (Fig. 4).

**Marimastat increases PPAR-α but not SREBP-1c levels.** Animals in the HCD+MAR group were found to have higher (151.7 ± 3.4) levels of PPAR-α than animals in both the HCD-only group (11.5 ± 4.4, \( P < 0.02 \)) and the control group (3.3 ± 1.7), as measured by Western blot densitometry (Fig. 5, A and B). No significant difference was observed between the nuclear and cytosolic protein components in densitometric analysis. The two values for each animal were summed and then subjected to statistical analysis. SREBP-1c protein levels were unchanged across the treatment groups.

**Marimastat increases phosphorylation of PPAR-α.** Immunoprecipitation followed by SDS-PAGE analysis revealed that PPAR-α in the control and HCD-only groups exists primarily in the unphosphorylated form. In contrast, the HCD+MAR group showed a marked increase in phosphorylation (Fig. 5C).

**Marimastat improves hepatic secretion of fat.** Hepatic triglyceride levels were substantially increased in HCD and significantly reduced by Marimastat, but triglyceride levels remained higher than in controls (Fig. 6A). There were no significant differences in hepatic phospholipids among the three groups. Serum triglyceride and phospholipids were significantly reduced in HCD-only compared with control (Fig. 6B) but were normalized with Marimastat treatment.

**Marimastat does not affect de novo lipogenesis.** Higher levels of palmitoleic acid (16:1) and oleic acid (18:1) reflect higher levels of de novo lipogenesis. Hepatic triglyceride and phospholipid levels of 16:1 and 18:1 were substantially elevated in both HCD-only and HCD+MAR compared with control (Fig. 6, D and E).

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Fig. 4. Serum IL-6 levels as measured by ELISA. Significant elevations in IL-6 levels were observed in the HCD+MAR group compared with the HCD-only group (\( P = 0.001 \)). HCD-only IL-6 levels were also significantly lower than the control group (\( P = 0.008 \)).

Fig. 5. Protein expression analysis of peroxisome proliferator activated receptor-α (PPAR-α) in control, HCD-only, and HCD+MAR groups. A: Western blot (SDS-PAGE) analysis of control and treatment groups with cytosolic (C) and nuclear (N) fractions, with increased expression in the HCD+MAR group. B: densitometric interpretation of the Western blot showing a significant increase of PPAR-α in the HCD+MAR group. C: Western blot (SDS-PAGE) analysis following immunoprecipitation showing increased phosphorylation of PPAR-α in the HCD+MAR group. D: densitometric interpretation of the Western blot shown as mean values with standard deviation and associated \( P \) value.
and clearance of fat from the liver (16). More specifically, the hit can result from an imbalance in the rate of entry, synthesis, and oxidation of fatty acids, usually occurring as a result of a high dietary fatty acid content, would exacerbate inflammation owing to its central role in inflammatory eicosanoid production. The first hit can result from an imbalance in the rate of entry, synthesis, and clearance of fat from the liver (16). More specifically, the uptake of triglycerides and fatty acids, the rates of resynthesis and de novo lipogenesis of fatty acids and triglycerides, the secretion of these compounds via plasma or bile, or oxidation of fatty acids may be altered (10, 17). Of these three basic processes there was no evidence for changes in fat synthesis in the HCD-only vs. the HCD+MAR diets, since SREBP-1c levels were unchanged in all three groups, and de novo lipogenesis appeared equally increased in both group as evidenced by free fatty acid profiles of liver triglycerides and phospholipids. There was evidence for improved clearance through secretion in HCD+MAR compared with HCD-only shown by increased serum triglyceride and phospholipid levels. IL-6, which promote hepatic clearance of lipid, were also dramatically increased in HCD+MAR.

PPAR-α is important in β-oxidation of fatty acids. An increase in oxidation would decrease the fat content of the liver. PPAR-α agonists including ciprofibrate, fenofibrate, and gemfibrozil reduce the secretion of TNF-α and increase fatty acid oxidation (19). We determined the PPAR-α protein expression in all groups and found a marked elevation (16-fold) of the PPAR-α protein in the HCD+MAR treated group. SREBP-1c is a protein that controls the transcription and expression of lipogenic enzymes including fatty acid synthase and stearoyl-CoA desaturase (8, 28, 55). It regulates the synthetic rate of triglycerides and the amount of storage in the liver (54, 56, 66), and inhibits lipogenesis. In all groups, SREBP-1c levels remained constant. This suggests that one of Marimastat’s principal mechanisms to decrease HCD-induced steatosis is through PPAR-α (increased beta oxidation) rather than decreased de novo lipogenesis. Furthermore, the data suggests that SREBP-1c may have limited impact on de novo lipogenesis.

In addition to increased protein expression, as shown by Western blot analysis, Marimastat increased phosphorylation of PPAR-α (Fig. 5C). The phosphorylated protein is the more active form of this protein (44). Thus Marimastat increases not

DISCUSSION

NAFLD is recognized as a major cause of liver-related morbidity and mortality with an ever-increasing socioeconomic impact. NAFLD presents as a variety of pathological states ranging from the simple buildup of fat in the liver (hepatic steatosis) to nonalcoholic steatohepatitis, cirrhosis, and ultimately liver failure (4, 45, 58). The etiology and potential therapy for this spectrum of pathologies has still not been fully elucidated. NAFLD is also associated with metabolic disorders such as obesity (46, 49, 60), diabetes (13), prolonged use of parenteral nutrition (7, 29, 52, 53), and chemotherapy (36, 50). Hepatic steatosis can be attributed to an increase in the synthesis of fat, a decrease in the oxidation of fat, or a decrease in fat secretion from the liver or some combination of these processes. A major stimulator of lipogenesis is a high-carbohydrate diet, and in particular one with simple sugars, because insulin, the prime lipogenic hormone, is maximally stimulated by glucose. A low-fat diet can further promote lipogenesis, since polyunsaturated fats particularly can reduce SREBP-1c levels that promote lipogenesis. Essential fatty acid deficiency further promotes lipogenesis and particularly de novo lipogenesis in which carbohydrate is both the source of the glycerol and of the fatty acid in triglyceride production.

Several hypotheses have been proposed to explain the pathogenesis of steatosis and steatohepatitis, although to date none have been conclusive. One theory is the “two hit” hypothesis, in which the first hit involves the development of hepatic steatosis (58), rendering the liver more susceptible to a second hit, resulting in more severe liver damage (17, 40, 59). Steatosis, particularly when arachidonic acid is a prominent stored fatty acid, usually occurring as a result of a high dietary linoleic acid content, would exacerbate inflammation owing to its central role in inflammatory eicosanoid production. The first hit can result from an imbalance in the rate of entry, synthesis, and clearance of fat from the liver (16). More specifically, the

Fig. 6. Triglycerides (TG) and phospholipids (PL) in liver and serum. A: total triglycerides and phospholipid level in liver. B: total triglycerides and phospholipid level in serum. C: Mead acid-to-arachidonic acid ratio in serum lipids. D: levels of 16:1 in liver. E: levels of 18:1 in liver. Total fatty acid levels in the liver go down with Marimastat treatment without correcting levels of mono-unsaturated fatty acids and Mead acid in the serum, indicating that Marimastat does not correct the essential fatty acid deficiency in mice on HCD.

A

B

C

D

E

A

B

C

D

E
only expression but also activation of PPAR-α, which may further facilitate β-oxidation of hepatic fat and reverse or prevent steatosis.

PPAR-α also reduces the secretion of TNF-α (19). A second mechanism of TNF-α reduction is through TACE. Marimastat is known to be a potent TACE inhibitor and therefore reducing the cell surface release of TNF-α may contribute to the amelioration of hepatic steatosis in our model, although the modest differences seen in TNF receptor II levels in this study would presumably have minimal impact on lipid synthesis.

Proinflammatory cytokines, in particular TNF-α, produced by Kupffer cells, appear to be involved in both the first and second hit of liver injury (37, 59). During the early stages, hepatocytes become exposed to TNF-α and Fas ligands. These molecules activate caspase pathways that lead to cell death (22). TNF-α itself is instrumental in the production of insulin resistance (15, 48). Hepatocyte exposure to TNF-α initiates intracellular signals that may lead to the release of reactive oxygen species and increased mitochondrial permeability (4, 39). Healthy hepatocytes respond to this insult by activating transcription factors such as nuclear factor-κB and adaptor protein 1 that can lead to adaptation and survival by synthesizing anti-apoptotic proteins such as Bfl-1 and oxidant-detoxifying enzymes such as manganese superoxide dismutase (21). Less viable, fatty, hepatocytes, however, are not as resilient and may react to TNF-α by activating the caspase pathway leading to apoptosis (22, 23) or by inducing sphingomyelinase and thereby increasing lipid peroxidation resulting in hepatocyte necrosis.

Animal models of liver injury include the mouse model of TNF-α infusion. Infusion of TNF-α produces rapid liver failure and death (64). This is prevented by the pretreatment of these animals with broad-spectrum MMP inhibitors. TNF-α antibodies in rats attenuate ethanol, and parenteral nutrition induced hepatic steatosis (30, 43). Broad-spectrum synthetic MMP inhibitors are also hepatoprotective in liver ischemia perfusion injury without a clear mechanism (14). We have developed an animal model (2, 3, 31) that utilizes a high-carbohydrate, no-fat diet that produces essential fatty acid deficiency. These animals develop steatosis and liver enzyme abnormalities in 19 days.

De novo lipogenesis is further stimulated by the inflammatory cytokine TNF-α. Release of soluble TNF-α is mediated by endogenous metalloproteinase activity. Liver injury is in part mediated by dysregulated matrix protein turnover that ultimately results in cirrhosis. Based on this information, our hypothesis was that MMPs may play a role in liver injury. To test this, we used a synthetic MMP inhibitor, Marimastat, in our model of steatosis. Our data show not only the prevention of steatosis, but also normalization of histology and liver enzymes. Although TNF-α receptor II levels were decreased, the principal changes were that IL-6 and PPAR-α levels were elevated. IL-6 is a hepatoprotective cytokine that at physiological and pharmacological doses increases liver fatty acid secretion, thereby reducing the total liver fat. The data support this mechanism. Although the markedly elevated PPAR-α levels are consistent with increased fatty acid oxidation as another major contributor to the amelioration of hepatic fat accumulation, further study will be necessary to document this process directly.

It is currently thought that inflammation plays a major role in the pathogenesis of fatty liver disease, and an important role for TNF-α has been suggested. This inflammatory cytokine is initially expressed as a 26-kDa cell-associated preform that is proteolytically processed to a 17-kDa soluble mature form by TACE, a member of the metalloproteinase family. It has previously been reported that inhibitors of MMPs inhibit the release of this mature form of TNF-α both in vitro and in vivo (18, 25, 38, 51, 64). We therefore hypothesized that inhibition of MMPs may prove beneficial in preventing hepatic steatosis, the first feature of NAFLD, in a murine model. The dramatic reduction of fat content and improvement of liver function tests suggested that the mechanism of action is likely related to fatty acid metabolism, but it is unlikely that TNF-α played a major role in this model.

These studies present, for the first time, intriguing evidence that the use of a broad-spectrum MMP inhibitor may be effective in the inhibition of hepatic steatosis and suggest that this therapy may be a useful tool in the treatment of NAFLD. Future study should document the possibility of increased fat oxidation in dietary-induced hepatic steatosis induced by Marimastat both by indirect calorimetry and direct assessment of fat oxidation using tracer technology.

GRANTS

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