Rosiglitazone Versus Rosiglitazone and Metformin Versus Rosiglitazone and Losartan in the Treatment of Nonalcoholic Steatohepatitis in Humans: A 12-Month Randomized, Prospective, Open- Label Trial

Dawn M. Torres,[1] Frances J. Jones,[2] Janet C. Shaw,[3] Christopher D. Williams,[2] John A. Ward,[4] and Stephen A. Harrison[5]

Medication combinations that improve the efficacy of thiazolidinediones or ameliorate weight-gain side effects of therapy represent an attractive potential treatment for (NASH). The aim of this randomized, open-label trial was to assess the efficacy of rosiglitazone and metformin in combination versus rosiglitazone and losartan, compared to rosiglitazone alone, after 48 weeks of therapy. A total of 137 subjects with biopsy-proven NASH were enrolled and randomly assigned to receive either 4 mg twice-daily of rosiglitazone, 4 mg of rosiglitazone and 500 mg of metformin twice-daily, or 4 mg of rosiglitazone twice-daily and 50 mg of losartan once-daily for 48 weeks. Patients were screened for other etiologies of chronic liver disease, including daily alcohol intake in excess of 20 g. Repeat liver biopsy was performed after 48 weeks of therapy and reviewed in a blinded fashion by a single expert hepatopathologist. The primary aim of the study was to assess for differences between treatment groups in the improvement of steatosis, hepatocellular inflammation, and fibrosis. In total, 108 subjects completed the trial. Primary outcome revealed no significant difference between treatment groups in all histologic parameters (steatosis, $P = 0.137$; hepatocellular inflammation, $P = 0.320$; fibrosis, $P = 0.229$). Overall improvement in steatosis, hepatocellular inflammation, ballooning degeneration, and fibrosis was observed ($P \leq 0.001$). Serum aminotransferases were reduced in all three groups ($P < 0.001$ within treatment, $P > 0.05$ between groups). Metformin did not significantly mitigate weight gain ($P = 0.051$). *Conclusions:* Forty-eight weeks of combination therapy with rosiglitazone and metformin or rosiglitazone and losartan confers no greater benefit than rosiglitazone alone with respect to histopathology. (HEPATOLOGY 2011;54:1631-1639)

See Editorial on Page 1503

Nonalcoholic fatty liver disease (NAFLD) and its most clinically relevant subset, those with nonalcoholic steatohepatitis (NASH), are a growing health concern throughout the world. The clinical importance of NAFLD is well established and extends beyond primary liver disease to include higher rates of cardiovascular and other metabolic complications and increased overall mortality.[1-4]

The ideal treatment for NASH has yet to be determined. Obesity and insulin resistance are inextricably linked to NASH, and, therefore, therapies directed at

Abbreviations: ARB, angiotensin receptor blocker; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAMC, Brooke Army Medical Center; BMI, body mass index; FDA, the U.S. Food and Drug Administration; HOMA-IR, the homeostasis model assessment for insulin resistance; NAFLD, nonalcoholic fatty liver disease; NAS, Nonalcoholic Fatty Liver Disease Activity Score; NASH, nonalcoholic steatohepatitis; SNPs, single-nucletide polymorphisms; TZD, thiazolinedione.

From the [1]Division of Gastroenterology, Department of Medicine, Walter Reed Army Medical Center, Washington, DC; [2]Division of Gastroenterology, Department of Medicine, Wilford Hall Medical Center, San Antonio, TX; [3]Department of Pathology, Wilford Hall Medical Center, San Antonio, TX; [4]Department of Clinical Investigation, Brooke Army Medical Center, Fort Sam Houston, TX; and [5]Division of Gastroenterology, Department of Medicine, Brooke Army Medical Center, Fort Sam Houston, TX.

Received May 4, 2011; accepted July 1, 2011.

The opinion or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the view of the U.S. Department of the Army or the U.S. Department of Defense.

The study protocol is registered at ClinicalTrials.gov: NCT00699036.

weight reduction and improved insulin sensitivity have been investigated. Lifestyle modification is currently the initial recommendation, but short of recommending diets void of processed sugars and saturated fat,[5,6] the ideal diet is not known. Recently, cross-sectional, self-reported data from a large cohort of well-defined NAFLD patients demonstrated that only vigorous exercise ($\geq$75 minutes/week) was associated with a decreased likelihood of having NASH.[7] Lifestyle modification may be limited in its clinical utility, because patients often cannot maintain either dietary changes or exercise habits.

Pharmacotherapy is widely accepted for many chronic medical conditions, and agents that improve hepatic histology would be quite useful. The thiazolinedione (TZD) class of insulin sensitizers have shown variable efficacy in NASH, but are limited by side effects, such as weight gain and, possibly, osteopenia and cardiovascular disease.[8-11] Although data suggest that up to 47% of patients resolve NASH with pioglitazone, improvement in hepatic fibrosis has shown modest, if any, improvement with TZD therapy.[8-11] In three previous studies with pioglitazone, fibrosis improvement was observed in 29%-46% of patients, but was not significantly different than placebo.[8-10] In a previous study with rosiglitazone, 16% of patients showed improved fibrosis and only 3% worsened, compared with 16% and 19%, respectively, for placebo.[11]

Given the modest effects of TZDs on NASH resolution and fibrosis regression, studies aimed at combination therapies that synergistically improve insulin resistance seemed laudable. Two medications thought to be good candidates for combination included the biguanide, metformin, and the angiotensin receptor blocker (ARB) class of drugs. In addition to the positive benefit of modest weight loss with metformin, data suggest an improvement in serum aminotransferases when used as monotherapy in NASH patients. Unfortunately, metformin has not been shown to significantly improve hepatic histology as monotherapy in the treatment of NASH.[12,13] ARBs were developed as antihypertensive medications that now have utility in a variety of diseases, including heart failure and chronic kidney disease. This versatile therapeutic drug class may also have utility in NASH, because early evidence suggests that they may improve insulin resistance[14,15] and hepatic fibrosis.[16]

Subsequently, this study was designed to compare the therapeutic efficacy of three treatment regimens in biopsy-proven NASH patients: rosiglitazone alone, rosiglitazone plus metformin, and rosiglitazone plus losartan.

Patients and Methods

Patients were recruited for this randomized, open-labeled, prospective, clinical trial from March 2007 to March 2010 from the outpatient Hepatology and Gastroenterology Clinics at Brooke Army Medical Center (BAMC; San Antonio, TX). The protocol was approved by the BAMC Institutional Review Board, and all subjects gave written informed consent. The study was investigator-initiated, and no funding was received.

Subjects. Patients 18-70 years of age with biopsy-proven NASH were enrolled. NASH was confirmed on liver biopsy by the presence of steatosis, hepatocellular inflammation, and hepatocyte ballooning degeneration, with or without fibrosis, utilizing the Brunt criteria.[17] Liver biopsy must have been within the 6 months before enrollment, or else a repeat-baseline liver biopsy was required to be eligible for enrollment.

Exclusion criteria included the following: patients with New York Heart Association class 3 or 4 heart failure, insulin-requiring diabetics, patients with a history of TZD, metformin, or ARB use in the 3 months before enrollment, alcohol consumption >20 g/day in a female and >30 g/day in a male, serum creatinine on initial screening of greater than 1.4, known hypersensitivity to a study drug, known history of diabetic ketoacidosis, and pregnant or breast-feeding females. Patients were also excluded if there was evidence of coexistent chronic liver disease to include viral hepatitis, Wilson's disease, autoimmune hepatitis, hemochromatosis, primary biliary cirrhosis, or primary sclerosing cholangitis.

Design. Eligible patients were randomly assigned using a computer-generated, random-sequence grid maintained by the principal investigator to one of three treatment arms: rosiglitazone 4 mg by mouth

Address reprint requests to: Stephen A. Harrison, M.D., Division of Gastroenterology and Hepatology, Department of Medicine, Brooke Army Medical Center, 3551 Roger Brooke Dr. 78234, Fort Sam Houston, TX. E-mail: Stephen.harrison@amedd.army.mil; fax: 210-916-3195.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.24558

Potential conflict of interest: Dr. Harrison advises Amylin. He received grants from Mochida and Rottapharm. Dr. Williams is on the speakers' bureau of Vertex and Kadman.

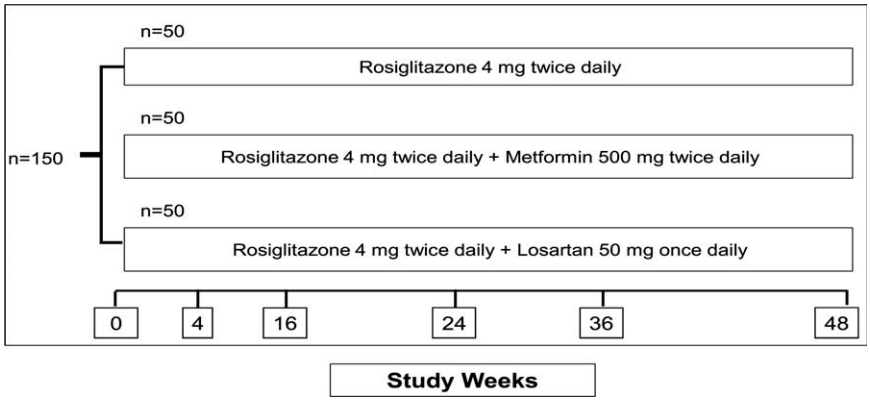

Fig. 1. Study design. Eligible patients were randomly assigned to one of three treatment arms: rosiglitazone 4 mg by mouth twice-daily, rosiglitazone 4 mg and metformin 500 mg by mouth twice-daily, or rosiglitazone 4 mg twice-daily and losartan 50 mg daily. Laboratory data were collected at 0, 24, and 48 weeks, consisting of fasting insulin level, fasting lipid panel, fasting glucose, hemoglobin A1c, C-reactive protein, basic metabolic panel, and liver function panel. A comprehensive metabolic panel was checked at 4, 16, and 36 weeks to monitor serum aminotransferase levels.

twice-daily, rosiglitazone 4 mg and metformin 500 mg by mouth twice-daily, or rosiglitazone 4 mg twice-daily and losartan 50 mg daily (Fig. 1). All medication doses were selected based on the usual starting does of these medications. All patients were counseled on reducing caloric intake and increasing physical activity as part of standard-of-care therapy for NASH patients. Alcohol use greater than 20 g/day in females and 30 g/day in females was assessed by direct questioning on the screening physical exam. Patients were counseled to limit their alcohol use to 1-2 drinks per week during the course of the study ,and this was reviewed during follow-up visits. Demographic data collected at screening included age, sex, and race. Weight, height, and vital signs were collected at screening and at end of the study. Body mass index (BMI) was calculated by weight in kilograms divided by the square of the height in meters. Blood pressure was recorded at the screening visit. Subjects enrolled in the rosiglitazone and losartan arm had a repeat blood-pressure check at 1 week into the protocol to evaluate for hypotension.

Laboratory data were collected at 0, 24, and 48 weeks, consisting of fasting insulin level, fasting lipid panel, fasting glucose, hemoglobin A1c, C-reactive protein, basic metabolic panel, and liver function panel. The homeostasis model assessment for insulin resistance (HOMA-IR) was used to calculate insulin resistance, according to the following formula: (milligrams of glucose per deciliter $\times$ microunits of insulin per milliliter) $\div$ 406. In addition, a comprehensive metabolic panel was checked at 4, 16, and 36 weeks to monitor serum aminotransferase levels.

An additional 5-mL serum aliquot was collected at weeks 0 and 48 and frozen for future analysis.

Patients were questioned regarding adverse events at every telephone encounter relaying laboratory results and at the time of requests for study-drug refills.

After 48 weeks of treatment, a repeat liver biopsy was performed to assess for improvement in histopathology. All liver biopsies were reviewed by a single expert pathologist in a blinded fashion. Liver biopsies were performed using a 14-gauge BARD® trucut needle with an average pre- and post-tissue length of 1.5 cm. Histopathologic parameters evaluated included the presence and degree of steatosis, hepatocellular inflammation, hepatocyte ballooning degeneration, Mallory-Denk bodies, and pericellular or other fibrosis. Hepatocellular inflammation and ballooning in the setting of steatosis were required to make the diagnosis of NASH. Steatosis with fibrosis alone or steatosis with inflammation alone did not qualify as NASH. Liver biopsies also were scored using the Nonalcoholic Fatty Liver Disease Activity Score (NAS), which assesses steatosis, inflammation, and ballooning degeneration with Mallory-Denk bodies.[18] Steatosis was graded as 0 for $<$5%, 1 for 5%-33%, 2 for 33%-66%, and 3 for $>$66% steatosis. Inflammation was graded as 0 for none, 1 for $<$2 foci per 20$\times$ field, 2 for 2-4 foci per 20$\times$ field, and 3 for $>$4 foci per 20$\times$ field. Hepatocellular ballooning degeneration was graded as 0 for none, 1 for mild/few, and 2 for moderate-marked/many. Fibrosis was staged as the following: stage 1 for zone 3 perivenular, perisinusoidal/pericellular fibrosis, focal or extensive; stage 2 for stage 1 disease with focal or extensive periportal fibrosis; stage 3 for bridging fibrosis, focal or extensive; and stage 4 for cirrhosis.

Primary and Secondary Outcomes. The primary outcome was a difference in the improvement of

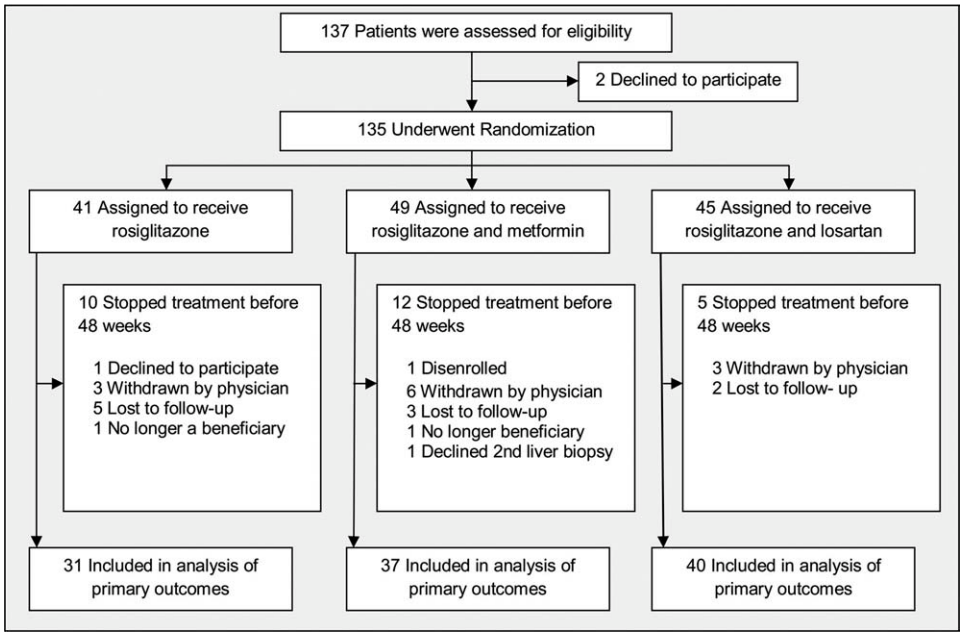

Fig. 2. Study-subject flow diagram for screening and randomization.

steatosis, hepatocellular inflammation, or fibrosis between treatment groups. A minimum 1-point improvement in each quartiled graded parameter was required to meet the primary end-point. Secondary outcomes included overall changes in steatosis, hepatocellular inflammation, hepatocyte ballooning degeneration, fibrosis, NAS, insulin, and alanine aminotransferase (ALT), as all three groups received rosiglitazone therapy. Changes in weight, other metabolic parameters, and other liver enzymes were additional secondary end-points.

Statistical Analysis. The primary analysis was a per-protocol analysis. Comparisons for primary and secondary outcomes were made using a two-factor analysis of variance (treatment, time), with repeated measures on one factor (time). Correlations were determined by linear regression analysis with backward elimination. Sample size was derived using a look-up table, based on employing the methods of Kraemer and Thiemann (1988), to obtain an initial sample size. The sample size was adjusted with 1,000 iterations of a Monte Carlo simulation until the power was between 80% and 85%, with a level of confidence of 95%. With 45 subjects per group, an 0.8 standard deviation would be detected between groups. An additional 5 patients were added to allow for dropouts.

Results

Study Subjects. In the fall of 2010, the U.S. Food and Drug Administration (FDA) restricted rosiglitazone to type II diabetics, prematurely halting the study at 137 patients enrolled. Of the 135 subjects that underwent randomization, 41 were assigned to receive rosiglitazone alone, 49 were assigned to receive rosiglitazone and metformin, and 45 were assigned to receive rosiglitazone and losartan (Fig. 2). Baseline characteristics were well matched between groups with respect to age, percent of diabetic subjects, gender, race, biochemical markers, metabolic factors, and histologic findings (Table 1). The difference in baseline NAS was significantly different ($P = 0.014$), with rosiglitazone alone having a higher baseline NAS, compared to the other two study groups. After a planned blinded, independent expert pathologist review at the completion of the study, 19 subjects were excluded based on the absence of stringent criteria for the diagnosis of NASH on their initial liver biopsy: 5 subjects (6%) in the rosiglitazone-alone arm, 9 subjects (19%) in the rosiglitazone and metformin arm, and 5 subjects (5%) in the rosiglitazone and losartan arm.

Primary Outcome. A total 108 subjects underwent an end-of-treatment liver biopsy. There was no statistically significant difference between rosiglitazone, rosiglitazone and metformin, and rosiglitazone and losartan with respect to improvement in steatosis (25%, 28%, and 25%; $P = 0.137$), hepatocellular inflammation (27%, 10.2%, and 19%; $P = 0.320$), or fibrosis (48%, 30%, and 18%; $P = 0.229$). When data were reanalyzed, eliminating the 19 patients without NASH at baseline, no differences, with respect to steatosis, hepatocellular inflammation, or fibrosis, were found between groups (Table 2).

Table 1. Baseline Characteristics of the Study Subjects*

Characteristic	Rosiglitazone (n = 31)	Rosiglitazone and Metformin (n = 37)	Rosiglitazone and Losartan (n = 40)	*P* Value
Demographic factors				
Age (yrs)	47.6 (± 9.0)	49.7 (± 10.4)	50.6 (± 10.6)	0.454
Diabetics (HgA1c ≥6.5) (%)	13	16	20	0.726
Gender (male) (%)	65	51	75	0.097
Race or ethnic group (%)†				0.283
Caucasian	65	76	55	
African American	0	5	5	
Hispanic	32	19	18	
Other	3	0	8	
Serum biochemical levels				
Alanine aminotransferase (U/L)	89.5 (± 51.6)	73.8 (± 64.1)	94.5 (± 76.0)	0.366
Aspartate aminotransferase (U/L)	54.7 (± 24.2)	52.0 (± 37.1)	60.1 (± 51.6)	0.671
Alkaline phosphatase (U/L)	92.1 (± 32.6)	96.1 (± 30.3)	86.9 (± 19.0)	0.335
C-reactive protein (mg/dL)	0.8 (± 1.0)	0.7 (± 0.6)	0.7 (± 0.6)	0.711
Lipids				
Triglycerides (mg/dL)	237.3 (± 264.9)	181.7 (± 98.7)	173.3 (± 106.8)	0.236
Total cholesterol (mg/dL)	214.8 (± 56.4)	208.5 (± 41.6)	201.2 (± 42.0)	0.474
Low-density lipoprotein (mg/dL)	133.8 (± 35.7)	127.2 (± 32.4)	126.7 (± 37.8)	0.659
Metabolic factors				
Weight (kg)	98.0 (± 18.9)	94.7 (± 20.9)	98.8 (± 23.2)	0.676
Body mass index (kg/m^2)‡	33.6 (± 4.1)	33.1 (± 6.4)	32.9 (± 5.9)	0.865
Fasting serum glucose (mg/dL)	100.5 (± 16.0)	106.7 (± 33.6)	103.6 (± 24.3)	0.625
Insulin (μIU/mL)	23.2 (± 15.8)	24.7 (± 19.3)	25.4 (± 12.8)	0.854
HgA1c (%)	5.8 (± 0.5)	5.9 (± 0.9)	6.0 (± 0.7)	0.317
Insulin resistance§	5.7 (± 4.5)	6.5 (± 5.4)	6.7 (± 4.2)	0.658
Liver histologic findings				
NAFLD activity score (NAS)‖	5.1 (± 1.4)	4.1 (± 1.6)	4.9 (± 1.2)	0.014
Steatosis	3.1 (± 0.9)	2.7 (± 0.9)	2.9 (± 0.8)	0.192
Lobular inflammation	1.6 (± 0.8)	1.3 (± 0.8)	1.6 (± 0.6)	0.129
Ballooning	1.2 (± 0.5)	1.0 (± 0.6)	1.2 (± 0.5)	0.141
Fibrosis stage¶	1.4 (± 1.0)	1.2 (± 0.9)	1.5 (± 1.0)	0.460
Absence of ballooning on central review	6%	19%	5%	0.229

*Plus-minus values are means ± standard deviation. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for glucose to millimoles per liter, multiply by 0.05551.

†Race or ethnic group was self-reported.

‡Body mass index is the weight in kilograms divided by the square of the height in meters.

§The homeostasis model assessment for insulin resistance (HOMA-IR) was used to calculate insulin resistance, according to the following formula: (milligrams of glucose per deciliter × microunits of insulin per milliliter) ÷ 405. Higher numbers indicate greater insulin resistance.

‖Total nonalcoholic fatty liver disease (NAFLD) activity score (NAS) was assessed on a scale of 0-8, with higher scores indicating more severe disease; the components of this measure include steatosis (assessed on a scale of 0-3), lobular inflammation (assessed on a scale of 0-3), and hepatocellular ballooning (assessed on a scale of 0-2).

¶Fibrosis was assessed on a scale of 0-4, with higher scores indicating more severe fibrosis.

Secondary Outcomes: Histologic Features. When the 89 patients with NASH on initial liver biopsy were analyzed for within-group comparisons, significant improvement was noted for steatosis, necroinflammation, ballooning degeneration, and fibrosis ($P \leq 0.001$) (Table 2). Improvement in fibrosis mildly correlated with low baseline values for steatosis (rho = −0.449), glucose (rho = −0.341), HOMA-IR (rho = −0.325), hemoglobin A1c (rho = −0.352), and triglycerides (rho = −0.315).

The NAS also improved significantly within all treatment arms. Good correlation (rho = 0.621) of the initial NAS with the change in NAS was noted. In general, the higher the initial NAS, the greater the change in NAS tended to be.

NASH resolution was seen in 36% of all patients, ranging from 29% in the rosiglitazone and losartan arm to 46% in the rosiglitazone alone arm. NASH resolution did not correlate with change in ALT level (rho = 0.042) nor HOMA-IR (rho = 0.140).

Clinical and Biochemical Parameters. Change in weight over the 48 weeks of therapy approached statistical significance between groups ($P = 0.051$). Both rosiglitazone only and rosiglitazone and losartan arms had a mean increase in weight (0.9 and 3.7 kg), whereas the rosiglitazone and metformin arm had a mean decrease in weight (−1.2 kg) (Fig. 3). BMI within groups showed significant difference with respect to time ($P = 0.018$). The rosiglitazone and losartan group was found to have a significant increase in

Table 2. Changes in Histopathology After 48 Weeks of Treatment

Variable	Rosiglitazone	Rosiglitazone and Metformin	Rosiglitazone and Losartan	*P* Value* Between Groups	*P* Value Overall
Number of subjects	26	28	35		
Changes from baseline in histologic features†					
Steatosis					
Subjects with improvement (%)	62	82	71		
Mean change in score	−0.85	−0.82	−0.74	0.905	<0.001
Lobular inflammation					
Subjects with improvement (%)	46	39	34		
Mean change in score	−0.58	−0.32	−0.34	0.46	<0.001
Hepatocyte ballooning degeneration					
Subjects with improvement (%)	42	39	34		
Mean change in score	−0.35	−0.18	−0.32	0.692	0.001
Fibrosis‡					
Subjects with improvement (%)	50	59	37		
Mean change in score	−0.7	−0.59	−0.32	0.302	<0.001
NAFLD activity score§					
Subjects with improvement (%)	77	71	71		
Mean change in score	−1.77	−1.32	−1.37	0.671	<0.001
Resolution of definite NASH (% of subjects)	46	36	29		

**P* values were calculated with the use of the two-factor analysis of variance (treatment, time) with repeated measures on one factor (time). There was no difference between groups for the primary outcome; however, study subjects receiving rosiglitazone statistically improved histologically overall.

†Listed percent subjects with improvement for each histologic feature of the liver required improvement by 1 or more points.

‡Fibrosis was assessed on a scale of 0-4, with higher scores indicating more severe fibrosis.

§Total nonalcoholic fatty liver disease (NAFLD) activity was assessed on a scale of 0-8, with higher scores indicating more severe disease; the components of this measure include steatosis (assessed on a scale of 0-3), lobular inflammation (assessed on a scale of 0-3), and hepatocellular ballooning (assessed on a scale of 0-2).

Abbreviation: NASH, nonalcoholic steatohepatitis.

BMI ($P = 0.001$, $P \leq 0.0167$; significant with Bonferonni's correction).

Significant improvement in glucose, insulin, and HOMA-IR ($P \leq 0.001$) (Table 3) was observed in all three groups. The addition of metformin did not improve fasting glucose, insulin, HOMA-IR, aspartate aminotransferase (AST), or ALT more than the other two arms ($P \geq 0.05$). Mean AST and ALT levels among all subjects significantly improved over the 48 weeks of therapy ($P \leq 0.001$) (Fig. 3).

Diabetics. Overall, 18 of the 108 subjects had diabetes, as noted by a hemoglobin A1c of ≥6.5 at time of enrollment, and were equally distributed in each arm (13% versus 16% versus 20%; $P = 0.726$). Diabetic subjects had a higher initial mean NAS, compared to nondiabetic subjects (5.75, 5.67, and 5.25 versus 5.0, 3.84, and 4.51). NAS significantly improved in diabetic subjects, compared to nondiabetic subjects ($P = 0.046$), with respect to treatment and time, but this appeared to be largely the result of improvement in steatosis ($P = 0.006$). No difference in NASH resolution was seen.

Adverse Events. Twenty-seven subjects did not complete the study. Ten patients were lost to follow-up. Two patients decided not to participate. Two patients separated from the armed services during the study and were no longer eligible for medical care. One patient declined a second liver biopsy. Twelve patients withdrew with the recommendation from their physician, 1 each for the following reasons: diarrhea, headache, nausea, fatigue, emotional liability, terminal prostate cancer, orthopedic injuries, hysterectomy, noncardiac chest pain, IgA nephropathy, lightheadedness, and weight gain.

Discussion

Insulin resistance is thought to be a key component in the pathogenesis of NASH. Consistent with this, across all treatment arms, patients treated with rosiglitazone improved their insulin sensitivity, reduced their serum aminotransferases, and showed benefits in hepatic histology. Histopathologic evidence of NASH resolved in 36% of cases, comparable to previous studies with pioglitazone.[8,10] Additionally, improvement in the NAS (71%-77%) was similar to our previous study with pioglitazone[10] and better than an earlier study done with rosiglitazone.[11]

Histopathologic improvements in some patients were observed, but universal improvement is lacking.

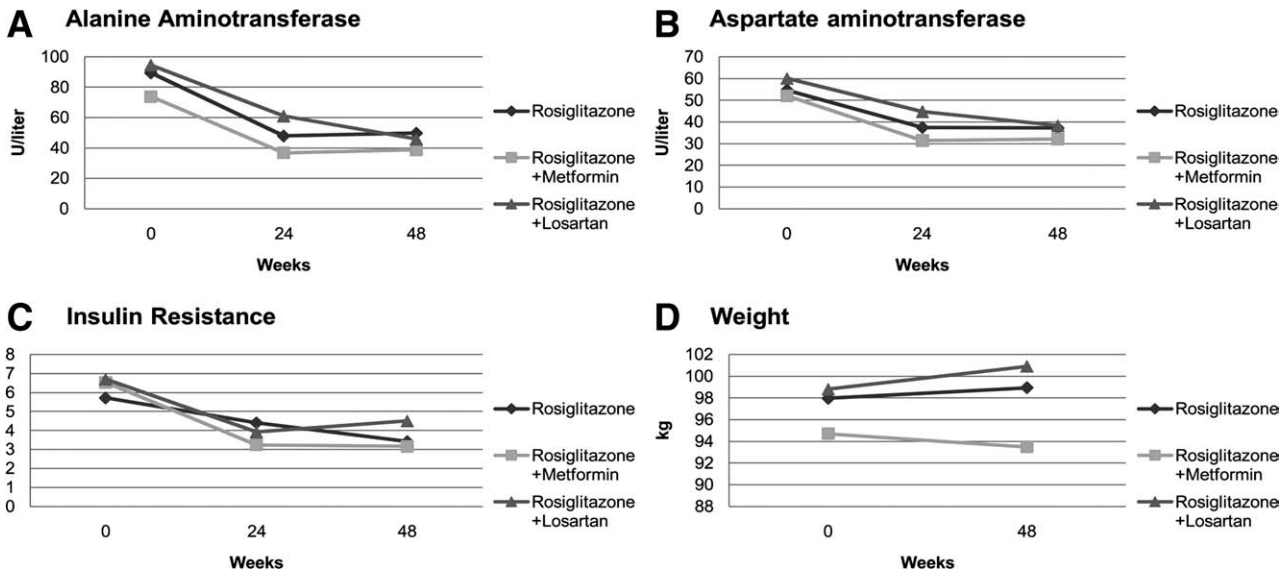

Fig. 3. Mean changes over time in aminotransferase levels, insulin resistance, and weight, according to study group. Mean values are shown for alanine aminotransferases levels (A), aspartate aminotransferases levels (B), insulin resistance (C), and weight (D) among the 31 subjects in the rosiglitazone group, 37 subjects in the rosiglitazone and metformin group, and 40 subjects in the rosiglitazone and losartan group. All available data were included in the calculation of means; data were missing for less than 10% of subjects. Insulin resistance was calculated according to the homeostasis model assessment, with the use of the following formula: (milligrams of glucose per deciliter x microunits of insulin per milliliter) ÷ 405.

Explanations for why more patients do not improve their histology or resolve NASH with TZD therapy are eagerly sought. The pathogenesis of NASH is likely much more complicated and multifaceted than what can be overcome with insulin-sensitizer therapy alone. Evidence from the PIVENS trial suggests a benefit from vitamin E, implying that oxidative stress may play more of a significant role in the pathogenesis of NASH than previously thought. Both environmental and genetic influences are also likely involved. For example, it has recently been shown that daily fructose consumption in middle-aged adults is associated with increased inflammation and ballooning degeneration, two histopathologic components that comprise the NAS and are required for the diagnosis of NASH.[19] Improvement in these variables with TZD therapy may have been abrogated in the setting of ongoing fructose ingestion that was not accounted for in this trial. Furthermore, genetic influences, in the form of single-nucleotide polymorphisms (SNPs), have recently been linked to hepatic steatosis and disease severity.[20-23] It is unknown whether these SNPs, or others yet unidentified, may impair histopathologic response to TZD therapy.

Table 3. Means and Changes in Means in Serum Biochemical Levels and Metabolic Factors*

Variable	Rosiglitazone (n = 31)	Rosiglitazone and Metformin (n = 37)	Rosiglitazone and Losartan (n = 40)	*P* Value† Overall
Serum biochemical levels				
Alanine aminotransferase (Δ)‡	49.9 (−39.6)	38.8 (−35.0)	45.8 (−48.7)	<0.001
Aspartate aminotransferase (Δ)	37.2 (−17.4)	32.1 (−19.9)	38.4 (−21.7)	<0.001
Alkaline phosphatase (Δ)	66.8 (−25.3)	66.0 (−30.1)	66.0 (−20.9)	<0.001
Metabolic factors				
Weight kg (Δ)	98.9 (0.9)	93.5 (−1.2)	100.9 (3.7)	0.269
Body mass index§	33.9 (0.3)	33.0 (−0.1)	33.7 (0.8)	0.018
Fasting serum glucose (Δ)	93.5 (−7.0)	96.8 (−7.9)	93.4 (−10.2)	<0.001
Insulin (Δ)	14.5 (−8.7)	13.0 (−11.7)	19.1 (−6.3)	<0.001
Insulin resistance (Δ)‖	3.4 (−2.3)	3.2 (−3.4)	4.5 (−2.2)	<0.001

**All available data were included in the calculation of means; data were missing for less than 10% of subjects.

†*P* values were calculated with the use of the two-factor analysis of variance (treatment, time) with repeated measures on one factor (time) (within-group comparison). There was no difference between groups.

‡Δ is the change in mean baseline and mean end-of-study value.

§The body mass index is the weight in kilograms divided by the square of the height in meters.

‖The homeostasis model assessment for insulin resistance (HOMA-IR) was used to calculate insulin resistance, according to the following formula: (milligrams of glucose per deciliter x microunits of insulin per milliliter) ÷ 405. Higher numbers indicate greater insulin resistance.

Unfortunately, our study did not show a benefit with the addition of metformin or losartan to rosiglitazone and leads to the conclusion that adjuvant therapies are thus ineffective. However, it is possible that the dose and/or type of concomitant study medication, and/or length of therapy, may have been incorrect. Metformin, though mitigating weight gain when added to rosiglitazone, was not associated with a significant improvement in insulin resistance, compared with the other arms. The dose of metformin was only 1,000 mg daily in this trial, and this may have been suboptimal, given that evidence suggests a dose response for plasma glucose and hemoglobin A1c up to a dose of 2,500 mg daily.[24]

Although the concept of ARB therapy to treat NAFLD is plausible, its effect, when added to rosiglitazone, was not evident in this study. Previous data have shown that in the setting of liver injury, the renin angiotensin system is activated and up-regulated, which contributes to the recruitment of inflammatory cells and activation of hepatic stellate cells.[25] In uncontrolled human trials, losartan was shown to down-regulate hepatic stellate cells[26] and improve serum aminotransferases as well as hepatic histology.[27] The apparent ineffectiveness of losartan in this study is disheartening, but does not rule out this class of medications as being potentially efficacious in NASH patients. Again, the dose of losartan may have been inadequate to have a synergistic effect. However, the study patients were not selected based on hypertension, and there was concern that treating normotensive patients with higher doses of losartan may have led to unintended negative consequences. Alternatively, in relation to NAFLD, it appears that other ARBs may be more efficacious than losartan. In rat models, telmisartan has shown comparable efficacy to pioglitazone in improving hepatic steatosis, necroinflammation, and fibrosis.[28,29] This is likely attributable to its pleiotrophic effects on peroxisome proliferator-activated receptor gamma up-regulation.[30] A subsequent trial by Georgescu et al. in 54 hypertensive NASH patients studied the effects of telmisartan 20 mg daily versus valsartan 80 mg daily for 20 months, and found that HOMA-IR significantly improved in both groups, but more so in the telmisartan group. Additionally, the NAS was significantly lower in the telmisartan group, compared with the valsartan group.[28]

The importance of liver biopsy to determine histologic change warrants mention. Changes in serum aminotransferases or insulin resistance did not predict histologic improvement and, therefore, should not be used as surrogate markers in evaluating the effectiveness of therapy. This underscores the need for pre- and postliver biopsy in all NASH clinical treatment trials, because proven, noninvasive tests to determine NAFLD severity are still lacking.

Limitations of this type of study are realized. The lack of blinding and a placebo control is apparent. However, given that four previously published double-blind, randomized, placebo-controlled trials with TZD therapy have already been completed and have generally shown benefit, it seemed unethical to treat biopsy-proven NASH patients with a placebo for 1 year and then subject them to a repeat liver biopsy. An additional limitation was the type of TZD chosen for this trial. During the course of this study, a black-box warning was issued by the FDA, and rosiglitazone use became quite restricted as a result of an association with myocardial ischemia.[31] Subsequently, study enrollment was halted at 137 patients, short of the enrollment goal of 150. Because of additional patient dropout, the possibility of a type II error cannot be excluded. Six patients enrolled in our study had to discontinue the study early as a result of this occurrence, but agreed to undergo repeat liver biopsies at the 6-month point. Two of these patients did not meet the strict histopathologic criteria for NASH on rereview by our hepatopathologist and thus were not included in the analysis. The remaining 4 patients improved their NAS by 1, 3, 4, and 7 points, respectively, and 3 of the 4 resolved NASH.

In conclusion, dual-agent therapy with metformin or losartan added to a rosiglitazone backbone did not improve histopathology, when compared to rosiglitazone alone. However, further study with an alternative ARB, such as telmisartan, or higher doses of metformin may be warranted.

References

1. Adams LA, Waters OR, Knuiman MW, Elliott RR, Olynyk JK. NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: an eleven-year follow-up study. Am J Gastroenterol 2009;104:861-867.
2. Yamada T, Fukatsu M, Suzuki S, Wada T, Yoshida T, Joh T. Fatty liver predicts impaired fasting glucose and type 2 diabetes mellitus in Japanese undergoing a health checkup. J Gastroenterol Hepatol 2010;25: 352-356.
3. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N Engl J Med 2010;363: 1341-1350.
4. Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005;129:113-121.
5. Yki-Jarvinen H. Nutritional modulation of nonalcoholic fatty liver disease and insulin resistance: human data. Curr Opin Clin Nutr Metab Care 2010;13:709-714.

6. Neuschwander-Tetri BA. Lifestyle modification as the primary treatment of NASH. Clin Liver Dis 2009;13:649-665.
7. Kistler KD, Brunt EM, Clark JM, Diehl AM, Sallis JF, Schwimmer JB;NASH CRN Research Group. Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. Am J Gastroenterol 2011;106:460-468; quiz, 469.
8. Sanyal AJ, Chalasini N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010;362:1675-1685.
9. Aithal GP Thomas JA, Kaye PV. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. Gastroenterology 2008;135:1176-1184.
10. Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med 2006;355:2297-2307.
11. Ratziu V, Giral P, Jacqueminet S, Charlotte F, Hartemann-Heurtier A, Serfaty L, et al. Rosiglitazone for nonalcoholic steatohepatitis: one-year results of the randomized placebo-controlled fatty liver improvement with rosiglitazone therapy (FLIRT) trial. Gastroenterology 2008;135: 100-110.
12. Bugianesi E, Gentilcore E, Manini R. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. Am J Gastroenterol 2005;100:1082-1090.
13. Uygun A, Kadayifci A, Isik AT, Ozqurtas T, Deveci S, Tuzun A, et al. Metformin in the treatment of patients with non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2004;19:537-544.
14. Aksnes TA, Reims HM, Guptha S, Moan A, Os I, Kjeldsen SE. Improved insulin sensitivity with the angiotensin II-receptor blocker losartan in patients with hypertension and other cardiovascular risk factors. J Hum Hypertens 2006;20:860-866.
15. Jin HM, Pan Y. Angiotensin type-1 receptor blockade with losartan increases insulin sensitivity and improves glucose homeostasis in subjects with type 2 diabetes and nephropathy. Nephrol Dial Transplant 2007;22:1943-1949.
16. Yokohama S, Yoneda M, Haneda M, Okamoto S, Okada M, Aso K, et al. Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis. Hepatology 2004;40: 1222-1225.
17. Brunt EM. Nonalcoholic steatohepatitis. Semin Liv Dis 2004;24:3-20.
18. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-1321.
19. Abdelmalek MF, Suzuki A, Guy C, Unalp-Aride A, Colvin R, Johnson RJ, et al. Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. Hepatology 2010;51:1961-1971.
20. Petersen KF, Dufour S, Hariri A, Nelson-Williams C, Foo JN, Zhang XM, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. N Engl J Med 2010;362:1082-1089.
21. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40:1461-1465.
22. Valenti L, Al-Serri A, Daly AK, Galmozzi E, Rametta R, Dongiovanni P, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin 148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2010;51:1209-1217.
23. Rotman Y, Koh C, Zmuda J, Kleiner DE, Liang TJ;NASH CRN. The association of genetic variability in PNPLA3 with histological severity of nonalcoholic fatty liver disease. Hepatology 2010;52:894-903.
24. Garber AJ, Duncan TG, Goodman AM, Mills DJ, Rohlf JL. Efficacy of metformin in type II diabetes: results of a double-blind, placebo-controlled, dose-response trial. Am J Med 1997;103:491-497.
25. Hirose A, Ono M, Saibara T, Nozaki Y, Masuda K, Yoshioka A, et al. Angiotensin II type 1 receptor blocker inhibits fibrosis in rat nonalcoholic steatohepatitis. Hepatology 2007;45:1375-1381.
26. Yokohama S, Tokusashi Y, Nakamura K, Tamaki Y, Okamoto S, Okada M, et al. Inhibitory effect of angiotensin II receptor antagonist on hepatic stellate cell activation in non-alcoholic steatohepatitis. World J Gastroenterol 2006;12:322-326.
27. Georgescu EF, Ionescu R, Niculescu M, Mogoanta L, Vancica L. Angiotensin-receptor blockers as therapy for mild-to-moderate hypertension-associated non-alcoholic steatohepatitis. World J Gastroenterol 2009;15:942-954.
28. Jin H, Yamamoto N, Uchida K, Terai S, Sakaida I. Telmisartan prevents hepatic fibrosis and enzyme-altered lesions in liver cirrhosis rat induced by a choline-deficient L-amino acid-defined diet. Biochem Biophys Res Commun 2007:364;801-807.
29. Fujita K, Yoneda M, Wada K, Mawatari H, Takahashi H, Kirikoshi H, et al. Telmisartan, angiotensin II type 1 receptor blocker, controls progress of nonalcoholic steatohepatitis in rats. Dig Dis Sci 2007;52: 3455-3464.
30. Yoshida T, Yamaqishi S, Matsui T, Nakamura K, Ueno T, Takeuchi M, et al. Telmisartan, an angiotensin II type 1 receptor blocker, inhibits advanced glycation end-product (AGE)-elicited hepatic insulin resistance via peroxisome proliferator-activated receptor-gamma activation. J Int Med Res 2008;36:237-243.
31. Rosen CJ. Revisiting the rosiglitazone story—lessons learned. N Eng J Med 2010;363:803-805.