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A Pilot Study of Pioglitazone for the Treatment of Non-Alcoholic Fatty Liver Disease

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Background and Aims: **Insulin resistance appears to be a major factor involved in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). In this pilot study, we examined the effect of pioglitazone, an insulin-sensitizing agent, on patients with NAFLD and NASH.**

Methods: **The medical records of patients referred to our clinic over a 48-month period were reviewed, and individuals with a clinical diagnosis of NAFLD or NASH, who were overweight (BMI≥25) with chronic elevated liver enzymes were included in this study. The patients were either treated with pioglitazone or advised to start a weight-reduction diet and exercise, in a non-blinded random method based on the treating physicians' discretion.**

Results: **Thirty-four patients' charts were retrospectively analyzed. Nineteen patients were treated with pioglitazone and 15 patients were advised to start a weight reduction diet and exercise. There were significant improvements in mean ALT and AST in the pioglitazone group at the end of treatment when compared to pretreatment values and to the diet/exercise group. There were no significant changes in the lipid profiles, body mass index or fasting glucose levels between baseline and at the end of the therapy in either group. There were no adverse side effects, including hypoglycemia, in patients treated with pioglitazone.**

Conclusions: **Preliminary results using pioglitazone in patients with NAFLD or NASH are promising. However, larger prospective studies are further needed to validate the results of our study and to examine histological response.**

Keywords: **NAFLD, NASH, Body Mass Index, Pioglitazone**

Introduction

Fatty liver may be present in up to 20% of obese individuals, with steatohepatitis found in 2-3% [1]. Since 20-25% of the US population is obese [2], fatty liver may represent the most common form of chronic liver disease in the Western world [3]. Weight loss and exercise in overweight individuals have been the only standard treatment for fatty liver disease. Weight reduction can improve liver chemistries, glucose tolerance, and free fatty acid elevations in proportion to the degree of weight loss [4]. However, the results of weight reduction and exercise therapy have been variable in the treatment of fatty liver in obese patients [5-11].

Non-alcoholic steatohepatitis (NASH) is characterized by the presence of histologic features similar to those of alcoholic hepatitis. However, patients with NASH do not have a history of significant alcohol consumption [12]. NASH is part of the spectrum of nonalcoholic fatty liver disease (NAFLD). This spectrum ranges from fatty liver alone to steatohepatitis in patients with NASH [13]. An elevated insulin level despite a normal blood glucose level characterizes the insulin resistance syndrome. Both NAFLD and NASH have been associated with the metabolic syndrome X that consists of insulin resistance, hypertension, diabetes mellitus type 2, hyperlipidemia, and obesity [14, 15].

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Insulin resistance appears to be a frequent finding in both the obese and non-obese patients with fatty liver disease and NASH [16]. This elevated insulin level inhibits the hepatic mitochondria's beta-oxidation of free fatty acids and decreases VLDL production. The resultant increase in free fatty acids in the liver stimulates the peroxisome proliferator activated receptors (PPAR). Fatty acids are then oxidized via P450 2E1 and peroxisomes leading to liver membrane lipid peroxidation [17]. The production of oxidized fatty acids also inhibits triglyceride export and stimulates the production of inflammatory cytokines with the ultimate recruitment of inflammatory cells. PPAR is also member of the steroid receptor family that modulates the transcription of a number of insulin responsive genes involved in the control of glucose and lipid metabolism [18].

It has been apparent that insulin resistance may be an important target for therapeutic intervention in patients with NAFLD/NASH. Pioglitazone, a third generation thiazolidinedione, is a potent and highly selective agonist of PPARγ that is commonly used in the therapy of type 2 diabetes mellitus. Pioglitazone acts primarily by decreasing peripheral insulin resistance as well as insulin resistance within the liver itself. Therefore, we wanted to determine the safety and efficacy of pioglitazone therapy in our patient population with liver disease due to NAFLD/NASH in comparison to the conventional advice for diet and exercise in an attempt to achieve weight reduction.

Materials and Methods

Patients and Study Design

This was a retrospective review of the medical records of patients referred with a clinical diagnosis of either NAFLD or NASH to the outpatient hepatology clinic at the University of Miami's Center for Liver Diseases over a period of 48 months. We wanted to compare patients treated with pioglitazone to the standard therapy of diet advice and weight loss. Individuals, who were at least 18 years of age, overweight (defined as a body mass index (BMI)≥25); with chronically elevated liver enzymes and a clinical diagnosis of NAFLD or NASH were included in our analysis. We were not able to clearly define the exact number of patients who had true NASH (i.e. with an active inflammatory component) versus the all encompassing diagnosis of NAFLD because all patients did not agree to undergo a baseline liver biopsy.

Each patient had an extensive evaluation to exclude other causes of chronic liver disease. All patients had negative viral hepatitis profiles (hepatitis A IgM, hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B core antibody, and hepatitis C antibody). All patients also tested negative for anti-nuclear, anti-smooth muscle, or anti-mitochondrial antibodies, and had normal iron, total iron binding capacity, ferritin and alpha-1-antitrypsin levels. Liver biopsies were performed to confirm the diagnosis and exclude all other causes of liver disease whenever possible. Histologic findings were graded and staged according to Brunt's classification [19]. No follow-up biopsies were performed for either group.

Interventions

Patients' charts were retrospectively reviewed. We found that patients had been randomly started on pioglitazone or were treated with the standard approach of encouraging exercise and a weight loss diet without any pharmacological intervention. The modality of therapy chosen was decided by the treating physicians' own discretion which was based on emerging data on the use of Thiazolidinediones in patients with NAFLD [20, 21] and after full discussion and consent of the patients. The weight loss diet consisted of a standard, restricted calorie, low fat diet. Pioglitazone was the only new therapeutic intervention during the study period. The starting dose was 15 mg orally per day. This dose was increased by 15 mg (maximum 45 mg/day) if liver enzymes did not improve by at least 50% after 8 to 12 weeks.

Endpoints

The primary endpoint of our study was improvement in the aminotransferases in comparison to their baseline values. Secondary end points were weight reduction and improvement in the lipid profile.

Statistical Analysis

The database of all patients that qualified for the study within the allotted period of time was collected with the Microsoft Excel program and formed the basis for the final analyses. Data were analyzed by STATISTICA program (Stat Soft, Tulsa, OK). Values were expressed as means±standard deviation. The student *t test* was used to test for significant differences between the two treatment groups as well as the pretreatment and post-treatment periods. P values less than 0.05 were considered significant.

Results

Baseline

Demographics and Syndrome X

Thirty-four patients were included in our study. Twenty-two patients were male and twelve were female with a mean age of 50.9 (range 24-81 years). All patients were overweight with body mass index (BMI)≥25. The mean BMI of the total group was 32.8 (range 25-43.7). Sixteen patients had evidence of insulin resistance (fasting insulin>14). Three of these 16 patients had type 2 diabetes mellitus. The insulin levels of 10 were not available, but 4 of these 10 patients had overt type 2 diabetes mellitus. A total of 7 patients had type 2 DM, all of whom received pioglitazone. Nine patients had essential hypertension and 8 had been treated with pioglitazone. For pretreatment patient demographics by treatment group see Table 1. The mean follow up period was 12.6 months (range 2-48 months).

Table 1. Pre-treatment patients' demographics and syndrome X characteristics.

	Diet	Pioglitazone	P value
Demographics			
Age	42.6±9.7	57.4±14.2	0.002
Gender (M/F)	12/3	10/9	NS
Syndrome X			
Weight (Kg)	89.7±15.1	92.7±20.7	NS
BMI	32.8±5.2	32.7±5.0	NS
DM (%)	0	37	NS
Fasting insulin levels	44.0	61.3	NS
HTN (%)	7	42	0.009

Values are presented as means±standard deviations; M/F: male/female; BMI: body mass index; DM: diabetes mellitus; HTN: hypertension; NS: non significant

Histology

Overall, 23 patients had a baseline liver biopsy prior to starting their particular treatment. In the diet treated group, 8 underwent a liver biopsy. Five of the 8 patients had a biopsy diagnosis consistent with NASH and 3 had steatosis alone (NAFLD) on biopsy. In the pioglitazone group, 15 patients had a biopsy. All 15 biopsies demonstrated changes consistent with NASH. Four of these 15 patients had cirrhosis in addition to NASH on their initial biopsy. In the remaining 4 patients in the pioglitazone group who did not have a biopsy, all 4 patients had at least 3 features of the metabolic syndrome X (some combination of obesity, hypertension, diabetes mellitus type 2, hyperinsulinemia, or hyperlipidemia).

Liver Function Tests and Lipid Profiles

There were no significant differences between the pretreatment liver function tests or the lipid profiles (total cholesterol, HDL, LDL or triglycerides) of the patients treated with diet alone when compared to the pioglitazone treated group at the baseline treatment period (Table 2).

Table 2. Pre-treatment liver function tests and lipid profiles.

	Diet	Pioglitazone	P value
Liver function tests			
Total bilirubin (mg/dl)	0.7±0.3	1.0±1.0	NS
Alkaline phosphatase (IU/L)	84±28	99±36	NS
ALT (x ULN)*	1.6±1.1	1.6±1.2	NS
AST (x UNN)*	1.2±1.0	1.7±1.0	NS
Total protein (g/dl)	7.8±0.4	7.8±0.8	NS
Albumin (g/dl)	4.5±0.2	4.0±0.6	0.01
Lipid panel			
Total cholesterol (mg/dl)	218±50	202±57	NS
HDL (mg/dl)	44±11	44±19	NS
LDL (mg/dl)	122±28	124±38	NS
Triglycerides (mg/dl)	208±132	180±105	NS

Values are presented as means±standard deviations.
*Times more than normal upper limit.

Treatment Outcomes

Patient Weights and BMI

There were no statistically significant changes in the post-treatment weights (diet: 91.4±14.7 kg; pioglitazone: 93.4±19.6 kg) or their BMI (diet: 33.0±4.6; pioglitazone: 33.3±4.9) of either the diet/exercise group or the pioglitazone group when compared to their pretreatment values (weights; diet: 89.7±15.1 kg, pioglitazone: 92.7±20.7 kg and BMI; diet: 32.8±15.1; pioglitazone: 32.7±5.0).

Liver Function Tests

In the diet/exercise group, 10 patients had a decrease in their ALT (10/15); however, this was not statistically significant. Eight patients had a decrease in their AST level (8/15), which again was not statistically significant. Furthermore, only 8 patients (8/15) were able to decrease both ALT & AST values at the same time with only one patient able to

normalize both values simultaneously.

In the pioglitazone group, 14 patients (14/19) had a statistically significant decrease in their ALT values (P=0.02). In addition, 16 patients (16/19) had a corresponding statistically significant decrease in their AST (P=0.005). Fourteen patients in the pioglitazone group were able to decrease both AST & ALT levels. Seven patients had both AST & ALT levels below the upper limits of normal at the end of treatment.

In the subgroup of 4 patients with biopsy proven cirrhosis who received pioglitazone, only 1 patient had an increase in ALT from (0.58 x ULN) to (0.71 x ULN) in parallel to an increase of AST from (1.45 x ULN) to (1.69 x ULN). The remaining 3 patients with cirrhosis treated with pioglitazone had marked improvements in both their AST & ALT levels.

Lipid Profiles

Statistical analysis failed to show any significant changes in post-treatment lipid profiles of either the diet or the pioglitazone groups when compared to their pretreatment values.

Adverse Experiences

There were no adverse experiences in either the dietary or the pioglitazone treatment groups. All patients tolerated the drug well, with none of the patients discontinuing its use because of adverse events. We did not see any hypoglycemia at the 15 mg per day dose of pioglitazone used in any of the patients that participated in this trial. There were also no patients with pioglitazone hepatotoxicity.

Discussion

To date, there is no standard approved treatment for patients with NAFLD/NASH. In the absence of FDA approved therapy, most physicians continue to recommend weight loss and exercise in these patients. Weight reduction can improve liver chemistries, glucose tolerance, and free fatty acid elevations in proportion to the degree of weight loss [4]. Unfortunately, the results of weight reduction and exercise therapy have been inconsistent in the treatment of NAFLD/NASH in obese patients and clearly cannot be used in patients that are at their ideal body weight [5-11].

Insulin resistance and hyperinsulinemia are strongly associated with fatty liver and NASH, even in non-obese patients [14, 16]. In the presence of peripheral insulin resistance, elevated serum insulin levels in NASH inhibits hepatic mitochondrial beta-oxidation of free fatty acids and VLDL secretion, resulting in increased levels of toxic free fatty acids in the liver. Accumulation of fatty acids leads to

Table 3. Diet group: Pre- and Post treatment liver function tests and lipid profiles.

Diet group	Pre-treatment	Post-treatment	P value
Liver function tests			
Total bilirubin (mg/dl)	0.7±0.3	0.7±0.5	NS
Alkaline phosphatase (IU/L)	84±28	85±26	NS
ALT (x ULN)*	1.6±1.1	1.2±0.7	NS
AST (x ULN)*	1.2±1.0	1.1±0.9	NS
Total protein (g/dl)	7.8±0.4	7.6±0.54	NS
Albumin (g/dl)	4.5±0.2	4.5±0.2	NS
Lipid panel			
Total cholesterol (mg/dl)	218±50	208±51	NS
HDL (mg/dl)	44±11	48±20	NS
LDL (mg/dl)	123±34	123±34	NS
Triglycerides (mg/dl)	208±132	216±201	NS

Values are presented as means±standard deviations.
*Times more than normal upper limit.

Table 4. Pioglitazone group: Pre- and post-treatment liver function tests and lipid profiles.

Pioglitazone	Pre-treatment	Post-treatment	P value
Liver function tests			
Total bilirubin (mg/dl)	1.0±1.0	0.9±0.8	NS
Alkaline phosphatase (IU/L)	99±36	92±33	NS
ALT (x ULN)*	1.6±1.2	0.8±0.5	0.02
AST (x ULN)*	1.7±1.0	1.0±0.4	0.005
Total protein (g/dl)	7.8±0.8	7.5±0.7	NS
Albumin (g/dl)	4.0±0.6	4.0±0.6	NS
Lipid panel			
Total cholesterol (mg/dl)	202±57	190±52	NS
HDL (mg/dl)	44±19	45±11	NS
LDL (mg/dl)	124±38	117±44	NS
Triglycerides (mg/dl)	180±105	132±67	NS

Values are presented as means±standard deviations.
*Times more than normal upper limit.

activation of the PPARs with oxidation of fatty acids via P450 2E1 and peroxisomes [17]. Products of lipid peroxidation inhibit the export of triglycerides and induce an inflammatory response. Increased oxidative stress leads to mitochondrial damage [22-26] and further accumulation of oxidized lipids in the hepatocyte [27].

PPAR is also member of the steroid receptor family that modulates the transcription of a number of insulin responsive genes involved in the control of glucose and lipid metabolism [18]. There are 3 types of PPARs receptors: alpha (α), beta (β), and gamma (γ). These receptors are found in tissues important for insulin action such as adipose tissue, skeletal muscle, and the liver. PPARα plays a role in determining the severity of hepatic steatosis [28] and its dysfunction in animals causes steatohepatitis [29]. This raises the possibility that abnormal peroxisomal responses contribute to fatty liver diseases in humans, including alcoholic liver disease and NASH. On the other hand, PPARγ also modulates the metabolism of glucose and lipids. In addition, it has been suggested that PPARγ may play a role in controlling the activation state of the satellite cells and, therefore, has a direct effect on hepatic fibrosis [17]. Activation of PPARγ nuclear receptors has been associated with numerous other effects in experimental conditions, such as increasing mitochondrial mass, reduction of circulating leptin, and inhibition of tumor necrosis factor alpha (TNFα) [18, 30]. Leptin and TNFα have also been implicated in the pathophysiology of NASH [31-33].

Insulin resistance, therefore, may be an important target for therapeutic intervention in patients with NASH. Metformin a drug that enhances insulin action on hepatocytes and decreases hepatic glucose production reversed fatty liver disease in obese leptin deficient mice [20]. Marchesini *et al*. followed 20 patients with NASH who were treated with metformin for 4 months. They found that metformin significantly decreased mean ALT concentration, significantly improved insulin resistance and decreased liver volume by 20% [34]. These findings are in contrast to those of Oruc and his group who found that metformin decreases AST but had no effect on insulin resistance and ALT levels [34]. Furthermore, metformin is known to induce lactic acidosis in some cases which could be life threatening.

Thiazolidinediones are anti-diabetic agents for type 2 diabetes mellitus that also act by improving insulin resistance. In one study, troglitazone (a thiazolidinedione) was administered for 6 weeks to Zucker diabetic fatty rats, which lack functional leptin receptors. At the end of the study, treated rats had a decrease in liver weight (32%), hepatic triglyceride content (62%), plasma triglycerides (80%), and plasma insulin levels (>50%) [20]. In another study, troglitazone administered for ≤6 months in 10 human subjects with NASH resulted in normalization of liver chemistries in 70% of the patients, and mild histologic improvement. Troglitazone, however, has been withdrawn from the market due to severe, idiosyncratic liver injury which has included liver failure leading to transplantation and death. Pioglitazone is another thiazolidinedione that has been shown to improve insulin resistance resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. In a pilot study by Kato *et al*, all 7 patients treated with pioglitazone for 3 months had normal ALT values at the end of the study period [21].

Pioglitazone is commonly used in the therapy of diabetes mellitus type 2. This third generation thiazolidinedione is a potent and highly selective agonist of PPARγ. Pioglitazone acts primarily by decreasing peripheral insulin resistance as well as that in the liver tissue, resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Therefore, we wanted to determine the safety and efficacy of pioglitazone therapy in our patient population with liver disease due to NAFLD/NASH in comparison to the conventional therapy of diet advice and weight reduction.

We found that in the group that had been treated with the conventional recommendation to lose weight and exercise, there was no statistically significant improvement in their liver function tests. There was also no significant (or even a trend toward) weight loss in the diet treatment group. This may explain their inability to achieve a statistically significant improvement in their liver function tests and clearly is a confounding variable in the evaluation of this treatment but, we feel that the lack of weight loss in the dietary/exercise treatment group (unfortunately) accurately mirrors the results seen in actual practice. In comparison, the group that had received pioglitazone significantly improved their aminotransferases values from their baseline values and induced normalization of aminotransferases in most patients. This appears to have been a direct result of the pioglitazone therapy since there was no decrease in the body mass index or a loss of weight while on pioglitazone. We also found it interesting that there was no significant change in the lipid profile in patients between baseline and at the end of the therapeutic period.

Rosiglitazone has been reported to cause severe reversible liver failure in two cases [35, 36]. A

potential concern with the use of pioglitazone for fatty liver is that it belongs to the same class of drugs as troglitazone and rosiglitazone and therefore, could potentially cause hepatotoxicity. Pioglitazone-associated liver toxicity was recently described in one patient with reversible asymptomatic aminotransferases elevation after 7 months of treatment [37]. Although our group of patients with cirrhosis was small, 3 out of 4 had a significant improvement in their liver function tests. Only one patient in this group of patients with cirrhosis that received pioglitazone had a 20% increase in their serum aminotransferases, but this was not accompanied by biochemical or clinical evidence of hepatic decompensation. Significant drug hepatotoxicity was not observed in any of our patients.

Pioglitazone has also been associated with side effects that include loss of appetite, vomiting, abdominal pain, diarrhea, bloating, lower leg edema, headache, anemia, or low white blood cell count in up to 5% of patients. In our study, all the patients tolerated the drug well, with none of the patients discontinuing its use because of adverse events. A final, obvious concern would be the development of hypoglycemia in those patients that were not diabetic and were treated with pioglitazone. Although this was a theoretical possibility, we did not see any hypoglycemia at the 15 mg per day dose of pioglitazone used in any of the non-diabetic patients that participated in this trial.

Our study was limited by the fact that it was an observational retrospective review of a small number of patients' medical records. Thus, it was not a double-blind randomized control study which has much more strength to its results. Furthermore, baseline liver biopsies were not carried out in all patients due to the patients' preference not to undergo a biopsy. Clearly, while improvements in liver function tests are important, the gold standard would be improved histology. Since this was a pilot trial, we did not feel that we could justify post-treatment liver biopsy without demonstrating at least biochemical improvement first. Another pitfall was the fact that the population studied was heterogeneous in terms of baseline co-morbidities (HTN, DM & insulin resistance) as well as baseline histopathology (fatty liver, NASH or cirrhosis), consequently response to treatment may slightly vary between one subgroup and another.

However, now that it has been shown that pioglitazone therapy can improve the aminotransferases, the next step will be to perform a prospective, double-blind, placebo-controlled study to see if there is an improvement in histology paralleling the biochemical improvement observed in this study, which should include pre- and post-treatment liver biopsies among a more homogenous group of patients.

In summary, we report our experience in treating our patient population with fatty liver, with pioglitazone compared to conventional therapy with dietary restriction and exercise. We found a significant improvement in the aminotransferases of the patients treated with pioglitazone compared to the group treated with a weight-loss diet and exercise. There were no side effects, including no episodes of hypoglycemia, in the patients that received pioglitazone. Pioglitazone appears to be a reasonable therapeutic agent to consider for prospective randomized trials to evaluate efficacy and safety in patients with NAFLD and NASH.

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