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Pentoxifylline for the treatment of non-alcoholic steatohepatitis: a randomized controlled trial(†)

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ABSTRACT

Introduction. The burden of non-alcoholic steatohepatitis (NASH) is growing and current pharmacologic treatments are limited by side effects and inconsistent efficacy. Pilot studies suggest that pentoxifylline (PTX) can reduce liver injury in patients with NASH. **Objective.** We sought to determine the tolerability of PTX and its effect on aminotransferases and liver histology in patients with NASH. **Material and methods.** Thirty patients with biopsy proven NASH were randomized in a 2:1 fashion to receive 1,200 mg PTX or placebo for 12 months. Metabolic parameters, aminotransferases, liver histology and hepatic gene expression changes were compared. **Results.** At baseline the groups were similar. Adverse events were mild, most frequently headache and abdominal cramps, and did not differ between groups (p = NS). After 12 months, ALT and AST decreased from 92 ± 12 IU/L to 67 ± 13 IU/L and 67 ± 6 IU/L to 47 ± 6 IU/L ($p < 0.05$), respectively in patients treated with PTX. No significant effect was seen with placebo. Steatosis and cellular ballooning improved in the PTX group ($p < 0.05$), whereas no histological feature of steatohepatitis improved with placebo. However, between groups comparison of both biochemical and histological features were nonsignificant. **Conclusion.** Pentoxifylline is safe, well tolerated and improves transaminases and histology in patients with NASH when compared to baseline and may be a reasonable therapeutic modality for the treatment of NASH. However PTX failed to reduce transaminases compared to placebo and did not positively affect any of the metabolic markers postulated to contribute to NASH. Although animal data and small pilot studies in humans have suggested that PTX may be effective as a treatment for NASH, translating this therapy to clinical practice may prove challenging.

Key words. Non-alcoholic fatty liver disease. Obesity. Cirrhosis. TNF alpha. Unfolded protein response.

INTRODUCTION

The obesity epidemic has increased the prevalence of obesity-related diseases such as non-alcoholic steatohepatitis (NASH), resulting in an increased number of patients undergoing liver transplantation for NASH cirrhosis (http://ustransplant.org). Current pharmacologic therapies for NASH can be divided into two main categories: insulin sensitizers and hepato-protectants. Given the complexity of this disease, it seems unlikely that a single pharmacologic therapy will suffice for the majority of patients. Recent data demonstrate that the insulin sensitizing thiazoledinediones (TZDs) are efficacious in reducing hepatic steatosis and liver injury in patients with NASH.[1-4] Although these results are encouraging, potential safety concerns have also been raised.[5]

Pentoxifylline (PTX) is a well tolerated drug used in patients with peripheral vascular disease that improves blood viscosity and erythrocyte rheological properties.[6] Furthermore, PTX is a nonspecific phosphodiesterase inhibitor that raises levels of cyclic adenosine monophosphate (cAMP) and de-

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creases tumor necrosis factor alpha (TNF-α) gene transcription.[7,8] We have previously shown that PTX attenuates liver injury in a murine model of NASH.[9] Uncontrolled pilot data in NASH showed a reduction in aminotransferases and an improvement in liver histology.[10,11] More recently, two small randomized trials suggest that PTX may reduce aminotransferases compared to placebo, however histologic outcomes were not examined.[12,13] Therefore, we conducted a randomized, double-blind, placebo-controlled proof-of-principle trial of 12 months of pentoxifylline versus placebo to determine efficacy in reducing aminotransferases as well as the histologic features of NASH.

The unfolded protein response (UPR) is an adaptive cellular response to various stressors. Recent data suggest that the activation and subsequent dysregulation of the UPR contributes to insulin resistance and inflammatory signaling in diabetes and may play an important role in human NASH.[14-17] TNF-α is known to directly activate the UPR and its downstream intracellular distress signals.[18] Thus, we postulated that PTX may modulate the UPR via a reduction in TNF-α activity.

MATERIAL AND METHODS

Patients

From March 2005 to March 2008 patients were recruited through the Northwestern Memorial Faculty Foundation Hepatology Clinic in Chicago, IL. The study was approved by the Institutional Review Board and Clinical Research Unit and posted on clinicaltrials.gov (NCT00267670). All patients with a diagnosis of NASH were evaluated for possible enrollment.

Inclusion and exclusion criteria

Subjects between the ages of 18 and 65 without evidence of other liver disease and an initial biopsy evaluation (within 6 months of entry) with steatohepatitis defined by the presence of steatosis, inflammation and ballooning were evaluated for inclusion into the study. Initial liver biopsy evaluation was performed by the local hepatopathologist.[19] Subjects with cirrhosis were allowed only in the setting of compensated liver disease. Other forms of liver disease were excluded serologically or with imaging as clinically indicated. All subjects included into the protocol were able to give informed consent.

Subjects were excluded from the study if they did not meet inclusion criteria or if they were HIV positive, pregnant or had evidence of ongoing alcohol consumption exceeding 20 g (males) and 10 g (females) daily. Furthermore, subjects were excluded if they were currently or within the preceding 6 months taking drugs known to cause steatohepatitis (i.e. tamoxifen, valproic acid, amiodarone, methotrexate, etc.). Those with current or a past history of decompensated liver disease, renal failure, evidence of active bleeding, cerebral or retinal hemorrhage were also excluded. Concomitant use of thiazolidenediones, weight loss medications, metformin, vitamin E, anti-TNF-α therapy or theophylline was prohibited. All patients on insulin secretagogues were excluded and no patients had addition of these medications during the study period. Lipid lowering drugs, sulfonylureas and insulin were allowed as long as the subject had been on a stable dose for 6 months and no dose adjustments were pursued during the study period.

Protocol

This was a prospective, randomized, double-blind, placebo controlled trial of patients diagnosed with NASH. Subjects were randomized to receive PTX 400 mg or placebo three times daily for 12 months. The primary goal of the study was to determine whether PTX therapy improved serum ALT (percent change from baseline to month 12) compared to placebo, based on expected improvement from the available literature at the time the trial was designed. Secondary endpoints were a decrease in non-alcoholic fatty liver disease (NAFLD) activity score (NAS), changes in the hepatic expression of inflammatory and UPR related genes, and serum cytokine levels.[20]

Assignment

A randomization table was generated to distribute groups in a 2:1 ratio. On the morning of the initial visit, subjects were randomized by the Northwestern pharmacy and supplied with corresponding pills (PTX or placebo).

Masking

Investigators and subjects were blinded to the treatment group. Placebo was identical in appearance to the study drug. The distribution sequence was only known to the research pharmacist and only unmasked at the end of the study to the study investigators.

Participant flow and follow-up

Although no specific dietary or exercise recommendations were given to either treatment group, diet and exercise were encouraged and monitored in all subjects. Compliance was assessed by history and confirmed by pill count at each visit. Subjects were seen at baseline, 3, 6, 9, and 12 months. At each visit, a history and physical examination was performed and anthropometric data and blood collected. All patients were asked to discuss any potential side effects during the study and specifically asked about the most commonly reported side effects (nausea, headache) seen in previous PTX studies. Side effects were noted if they occurred on more than one occasion. Care was taken to document the introduction of new medications or dose adjustments over the study period. Liver chemistries, renal function, lipid profile, blood counts, prothrombin time, insulin, adiponectin, leptin, TNF-α, and IL-6, IL-1β and IL-10 levels were measured. On the first and 12th month visit, all non-diabetic patients underwent an oral glucose tolerance test.

Analysis

- ***Biochemical endpoints.*** The primary endpoint was an improvement in ALT (percent change from baseline to month 12). ALT above a given threshold was not an entry criteria because the goal of the study was to assess the biological effect of PTX. Many patients with NASH have ALT in the "normal range", and lower cut-off values for "normal" have been proposed.[21,22] Therefore rather than only consider patients with ALT above an arbitrary value, we focused on the ability of PTX to lower ALT from its baseline starting value. At study completion, ALT levels from each visit were compared to baseline values in each treatment group. Serum cytokine levels before and after treatments were compared in each group in a similar fashion.

  ALT values were obtained from fresh serum. Insulin, leptin and adiponectin were determined using radioimmunoassays (Millipore, Billerica, MA), free fatty acid levels were measured using a Beckman Synchron CX-3. All other routine laboratory tests were performed by the Northwestern laboratory. Homeostatic Model Assessment (HOMA) score was calculated from fasting insulin (Ins_0, μU/mL) and glucose ($Gluc_0$, mg/dL) using the formula $Gluc_0 \times Ins_0/405$. Cytokines were measured using electrochemiluminescence detection technology (Meso Scale Discovery, Gaithersburg, MD).[23] The sandwich immunoassay format was adapted by multispot cytokine detection (MSD) plate for IL-1b, IL-6 and TNFα and labeled with electrochemiluminescent ruthenium complex (MSD Sulfo-TAG). Serum was added to the four-spot MSD according to the manufacturer's instructions. Cytokine levels were quantified on a MSD SECTOR Imager 2400.
- ***Histology.*** With the exception of subjects that had a liver biopsy elsewhere within 6 months of study entry, all liver biopsies were performed by the principal investigator (M.R.) and fixed in formalin. When possible, an additional core liver biopsy was obtained for gene expression analysis and placed in RNA*later* provided adequate tissue (≥ 2 cm in length) had been reserved for histology. Hematoxylin and eosin and Masson's trichrome stains were interpreted in a blinded fashion by a single hepatopathologist (E.B.). In addition to recording the individual histological features of steatohepatitis, a diagnosis was rendered: "definite NASH", "borderline NASH" or "not NASH" for each biopsy as previously described.[20] Since the diagnosis of steatohepatitis requires not only the presence of steatosis, lobular inflammation and ballooning, but also a zonal accentuation, borderline cases are those in which some, but not all features, are present. These results were compared for each patient at the end of the study.
- ***Histological endpoints.*** Individual components of the Brunt score and the composite NAS were compared in pre- and post-treatment liver biopsies.[24] When the study was designed, the NASH clinical research network (NASH CRN) classification system had not yet been published, thus the Brunt classification was used because it was the most well described classification system. During the study period the NASH CRN scoring system was published, thus a post hoc analysis using the NAS was also performed.[20]
- ***Genetic endpoints.*** Relative changes in gene expression using Real Time PCR were compared before and after treatment in each group. Results are reported as relative differences in gene expression compared to β-Actin (internal control). Total RNA was extracted from liver biopsy samples and cDNA synthesized in a systematic fashion as previously described.[9]
- ***Statistics and sample size.*** Descriptive statistics were provided for all variables described in the primary and secondary endpoints. Sample

size estimates were based on a 30% reduction in ALT in the treatment group and a 15% reduction in the placebo group. With a sample size of 30 planned (20 treatment:10 placebo), the study was designed to have a power of 90% to detect a difference in means of 1.25 standard deviations (ES = 1.25), and a power of 80% to detect a difference in means of 1.1 standard deviations (ES = 1.1), based on a calculation using power index, z-table, and accounting for unequal sample size: or $n_1 = 20$, $n_2 = 10$, and alpha = 0.05.

Data are presented as mean ± standard error (SE) for continuous variables. A paired student t test (normally distributed data) or Wilcoxon signed rank test (for data not normally distributed), was used to compare differences from pre- to post-treatment within a group. The primary efficacy endpoint (the percentage change from the baseline to month 12 ALT) was analyzed with a last observation carried forward algorithm for subjects who withdrew prematurely or missed an intermediate efficacy assessment. An analysis of covariance was used and

Table 1. Baseline patient demographics in all randomized NASH subjects.

	PTX pre-treatment (n = 21)	Placebo pre-treatment (n = 9)	p value
Age (years)	48 ± 2	53 ± 2	NS
Gender (F%)	62%	38%	NS
Race			NS
Caucasian	17	7	
Hispanic	3		2
Asian	1	0	
Cirrhosis	24%	25%	NS
Diabetes	10%	13%	NS
Other medications (%)			
Anti-diabetes	10%	12%	NS
Lipid lowering	14%	12%	NS
Body weight (kg)	97 ± 3	104 ± 10	NS
Waist/Hip ratio	0.95 ± 0.02	0.98 ± 0.02	NS
BMI (kg/m^2)	34.0 ± 0.9	35.1 ± 2.6	NS
Labs			
ALT (IU/L)	89 ± 11	80 ± 13	NS
AST (IU/L)	73 ± 11	64 ± 12	NS
INR 1.1	±0.3	1.1 ± 0.3	NS
Platelets	240 ± 18	223 ± 20	NS
Creatinine (mg/dL)	0.83 ± 0.04	0.97 ± 0.08	NS
Total cholesterol (mg/dL)	179 ± 7	186 ± 13	NS
Triglycerides (mg/dL)	151 ± 16	147 ± 29	NS
HDL (mg/dL)	37 ± 2	37 ± 2	NS
Free Fatty Acids (mEq/L)	0.78 ± 0.07	0.78 ± 0.08	NS
Leptin (ng/mL)	27 ± 3	23 ± 4	NS
Adiponectin (µg/mL)	5.8 ± 0.9	5.3 ± 0.9	NS
Insulin (uU/mL)	43 ± 9	33 ± 7	NS
Glucose (mg/dL)	108 ± 6	112 ± 5	NS
HOMA	8.9 ± 1.3	9.4 ± 2.4	NS
Liver histology			
NAFLD Activity Score	5.1 ± 0.3	4.0 ± 0.7	NS
Steatosis	1.8 ± 0.2	2.1 ± 0.3	NS
Lobular Inflammation	1.8 ± 0.2	0.9 ± 0.3	0.005
Ballooning	1.5 ± 0.2	1.0 ± 0.4	NS
Fibrosis	2.4 ± 0.2	1.8 ± 0.6	NS

Unpaired t-tests were used to compare the two treatment groups at baseline. Values represent relative means ± SEM.

the primary comparison of interest was the treatment group of PTX *vs.* the placebo. The percentage change from baseline to month 12 in proinflammatory cytokines, gene expression and histological endpoints were the secondary endpoints and were analyzed with the same analysis of covariance model and summary statistics specified for the primary endpoint. Differences were regarded as statistically significant when $P < 0.05$.

RESULTS

Baseline characteristics

The groups were well matched with respect to age, gender, and race, waist to hip ratio, body mass index (BMI) and degree of insulin resistance (defined as HOMA). Equal proportions of cirrhotic and diabetic subjects were enrolled in each group: 24 *vs.* 25% and 10 *vs.* 13% in the PTX *vs.* the placebo group, respectively. Furthermore, there were no differences in baseline laboratory values between the groups. The overall NAS, steatosis, ballooning and fibrosis scores were not different between the groups, although there was more lobular inflammation in the PTX group at study entry; 1.8 ± 0.2 *vs.* 0.9 ± 0.3 for PTX and placebo group, respectively ($p = 0.005$) (Table 1).

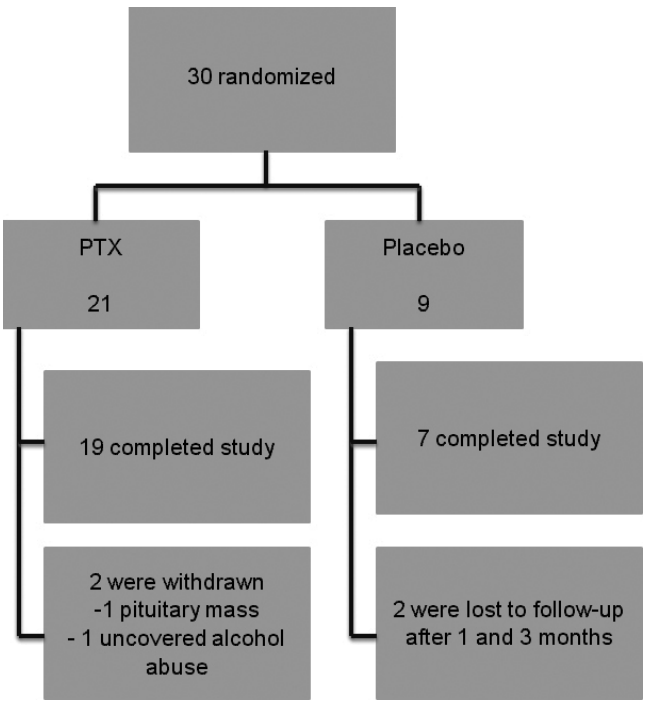

Figure 1. *CONSORT flow chart.*

Compliance and follow-up

Twenty six of 30 subjects enrolled, completed 12 months of therapy (19 PTX, 7 placebo). In the PTX group, 2 were excluded (discovery of a brain tumor and uncovered alcohol abuse). In the placebo group, 2 patients voluntarily withdrew and were lost to follow-up (Figure 1).

Of the 19 in the PTX group that completed 12 months of therapy, most (79%) consistently took 1,200 mg daily whereas 4 patients missed > 30% of the doses. All but one of the 26 subjects completing the study had a liver biopsy at month 12 (thrombocytopenia).

Clinical and biochemical parameters

Interval changes in serum aminotransferases during treatment at 3, 6, 9 and 12 months are summarized in table 2. Of those who completed 12 months of therapy (PTX 19, placebo 7), no changes in weight, BMI, serum aminotransferases or lipid parameters were noted between the groups. Fifty three percent of subjects in the PTX group achieved the predicted 30% reduction in ALT compared to only 14% in the placebo group, though this was not statistically significant (OR = 6.67, $p = 0.08$).

ALT and AST decreased from 92 ± 12 IU/L to 67 ± 13 IU/L and 67 ± 6 IU/L to 47 ± 6 IU/L after 12 months of treatment with PTX. No significant effect was seen in the placebo group in ALT; 77 ± 15 IU/L to 65 ± 13 IU/L or in AST; 64 ± 14 IU/L to 54 ± 10 IU/L for baseline compared to 12 months.

Of those subjects with elevated ALT on enrollment, 40% of subjects in the PTX group compared to 25% in the placebo group normalized their ALT by study completion, but this was not statistically significant across groups (OR = 2.0, p = NS). This improvement in aminotransferases generally occurred by month 3 and persisted throughout the study period. Although no significant decrease in weight was observed in either group, ALT improvement did correlate with weight loss; Spearman correlation coefficient 0.63 ($p < 0.001$).

Weight did not change in the PTX group; 97 ± 4 kg and 95 ± 4 kg at baseline and after 12 months, respectively or in the placebo group; 104 ± 11 kg at baseline to 103 ± 11 kg after treatment. Insulin resistance was not affected by PTX (Table 2). Treatment with neither PTX nor placebo had a significant effect on serum IL-1β, IL6 or TNF-α levels.

Table 2. Mean changes from baseline in patients who completed 12 months of therapy.

Parameter	PTX (n = 19)	Placebo (n = 7)	p value
Δ Weight (kg)	-2.2 ± 1.2	-0.9 ± 1.4	0.50
Δ ALT (IU/L)	-25.1 ± 10.3*	-12 ± 5.4	0.63
Mean (%) change in ALT			
3 months	-18.1 ± 6.3	-8.8 ± 20.3	0.98
6 months	-24.3 ± 5.6	-20.3 ± 12.8	0.47
9 months	-14.3 ± 12.9	-25.3 ± 7.7	0.36
12 months	-23.8 ± 8.3	-14.0 ± 7.4	0.69
Δ AST (IU/L)	-20.7 ± 7.9*	-10.1 ± 6.8	0.47
% normalization of ALT	6 (40%)*	1 (25%)	0.58
% normalization of AST	5 (33%)*	0 (0%)	0.69
Δ Insulin (μU/mL)	-11.9 ± 8.1	-5.6 ± 6.1	0.58
Δ Glucose (mg/dL)	-5.2 ± 4.9	11.3 ± 13.5	0.09
Δ HOMA	-1.3 ± 0.8	9.4 ± 2.4	0.56
Δ Leptin (ng/mL)	0.6 ± 1.8	0.7 ± 2.8	0.94
Δ Adiponectin (μg/mL)	0.4 ± 0.3	0.8 ± 0.4	0.49
Δ Triglycerides (mg/dL)	5.7 ± 19.2	-24.6 ± 29.8	0.32
Δ TNF-α (pg/dL)	-117.9 ± 109.5	18.3 ± 64.4	0.16
Δ IL-1β (pg/mL)	-7.8 ± 11.5	-4.3 ± 14.4	0.89
Δ IL-6 (pg/mL)	-22.1 ± 71.0	-82.9 ± 47.5	0.82
Δ Steatosis grade	-0.8 ± 0.2*	-0.6 ± 0.3	0.33
Δ Lobular inflammation	-0.1 ± 0.2	0.3 ± 0.3	0.97
Δ Hepatocyte ballooning	-0.5 ± 0.2*	0 ± 0.2	0.46
Δ Fibrosis score	-0.2 ± 0.3	0.4 ± 0.2	0.26
Δ NAS score	-1.4 ± 0.4*	-0.3 ± 0.4	0.17

Values represent relative means ± SEM. *Within group comparison was significant from baseline to 12 months, $p < 0.05$.

Histology

When comparing the effect of the drug within groups, steatosis grade improved in the PTX group from 1.8 ± 0.2 to 1.1 ± 0.2 ($p = 0.002$) with no significant improvement in the placebo group; 2.1 ± 0.3 to 1.6 ± 0.4 (p = NS). PTX reduced the degree of ballooning from 1.5 ± 0.2 to 1.0 ± 0.2 ($p < 0.05$) whereas no improvement occurred in those that took placebo (1 ± 0.4 to 1 ± 0.4). However, there were no significant differences in between group comparisons. In addition, no significant reduction was seen for lobular inflammation in either group.

Fibrosis scores, not included as secondary endpoints, likewise did not change significantly in either group. Figure 2 illustrates the change in histological scoring from baseline to 12 months of treatment with either PTX or placebo. A *post hoc* analysis was performed to evaluate the effect of PTX on the NAS. In subjects treated with PTX, the NAS decreased from 5.1 ± 0.3 to 3.7 ± 0.5 ($p = 0.002$). In contrast, no difference occurred after 12 months of placebo; 4.0 ± 0.7 *vs.* 3.7 ± 0.8.

When biopsies were given a diagnosis of either definite, borderline or not steatohepatitis, independent of the NAS, biopsy diagnosis improved as well in those that received PTX ($p = 0.008$). However, the proportion of subjects in the PTX *vs.* placebo group in whom biopsy diagnosis improved from borderline or definite steatohepatitis to not steatohepatitis was 44 *vs.* 28% (p = NS). No subject receiving PTX developed worsening steatohepatitis on post-treatment biopsy, though one subject receiving placebo worsened over time. Approximately 50% of subjects in both groups had no change in their overall diagnosis after treatment (p = NS).

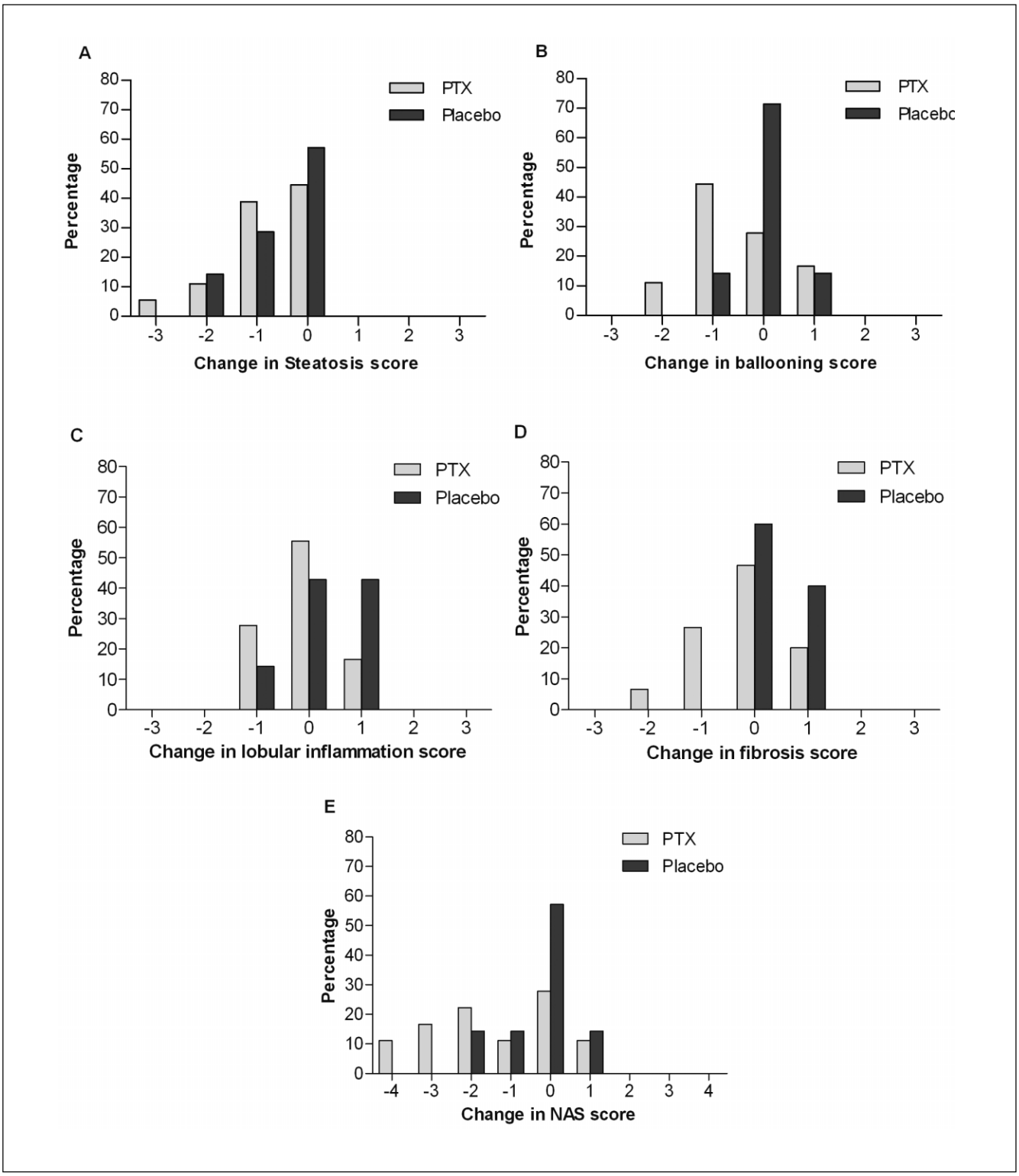

Figure 2. *Effects of pentoxifylline (PTX) or placebo on hepatic histology. Changes in histology in PTX and placebo treated patients after 12 months. Percentage of subjects without a change in histology were defined as "0", similarly, those whose histological score increased or decreased after treatment were noted with positive or negative numbers, respectively.* ***A.*** *Steatosis.* ***B.*** *Ballooning.* ***C.*** *Lobular inflammation.* ***D.*** *Fibrosis.* ***E.*** *NAFLD activity score.*

Hepatic fibrosis gene expression

Although histologically, no differences were seen following PTX or placebo on fibrosis scores, hepatic expression of collagen-1, a key gene involved in hepatic fibrogenesis, did decrease significantly in patients treated with PTX from 1.2 ± 0.2 to 0.5 ± 0.1 ($p < 0.03$). The change was not significant in the placebo group; 0.9 ± 0.1 at baseline to 0.6 ± 0.1 after 12 months (Figure 3). TIMP-1, another important fibrosis gene was reduced by 50% after PTX, though this did not reach statistical significance.

Hepatic expression of UPR genes

In the subset of 10 patients who had sufficient liver tissue available for analysis before and after treatment, hepatic expression of the UPR master regulator Bip was measured. After 12 months of therapy, Bip hepatic gene expression decreased by 70% in the PTX treated group ($p = 0.04$), suggesting an attenuation of UPR activation (Figure 3). A downward trend was also appreciated in CHOP, EDEM and GADD34 levels after PTX but not in placebo treated patients (data not shown).

Symptoms and tolerability

At baseline, right upper quadrant pain and fatigue were the most common complaints; 11 and 32% in the PTX group and 14% for both symptoms in the placebo group, respectively. After treatment this reduced to 5% for both pain and fatigue in the PTX group and to 14 and 16% in the placebo group, res-

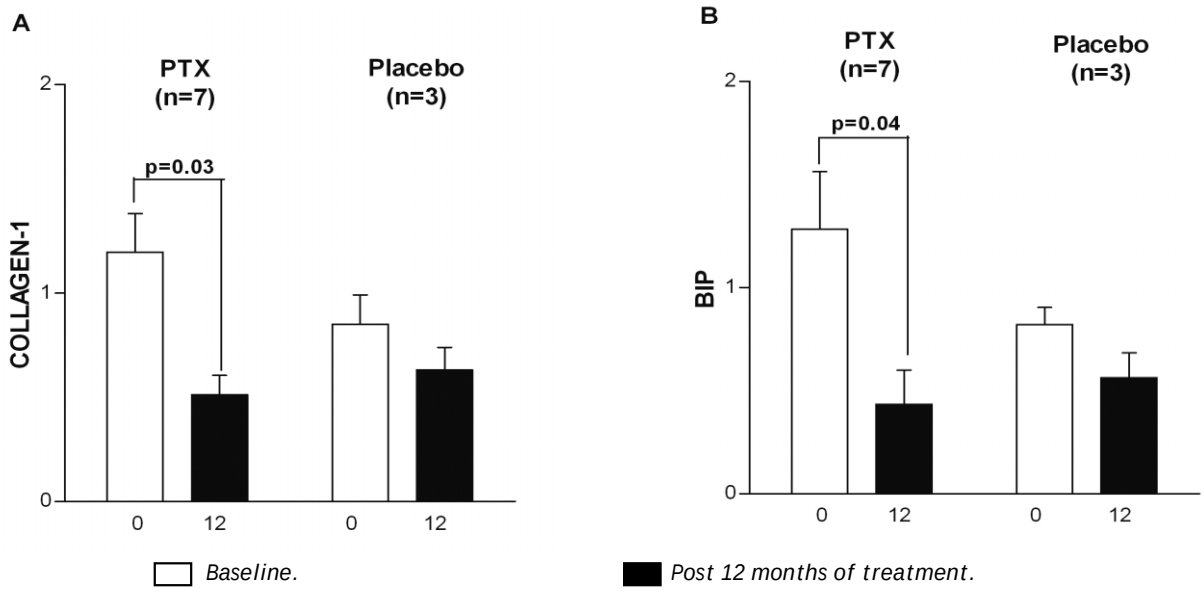

Figure 3. *Hepatic gene expression. Bars represent relative changes in hepatic gene expression of (**A**) collagen-1, or (**B**) Bip in patients treated with either pentoxifylline (PTX) or placebo for 12 monhts. Significance was defined as * p < 0.05.*

Table 3. Safety results.

Adverse events	Pentoxifylline month 12 (n = 19)	Placebo month 12 (n = 7)
Nausea x1/>1 time	n = 7(37%)/n=1	n = 1 (14%)
Abd. cramps	n = 4 (21%)	n = 3 (43%)
Bloat/Heart burn	n = 3 (16%)	n = 1 (14%)
Headaches	n = 3 (16%)	n = 1 (14%)
Fatigue/Insomnia	n = 0 (0%)	n = 0 (0%)
Serious adverse events	n = 0 (0%)	n = 0 (0%)

All adverse events (AEs) and serious adverse events (SAEs) were collected from the first dose of study medication to 30 days after the last dose. On-therapy AEs and SAEs were defined as any reported from the first dose of study medication until the last day of study medication.

pectively. None of these changes in symptoms reached statistical significance. Reported drug related side effects were mild and infrequent. The most common complaints were headache and abdominal cramps, which occurred at equal frequencies between the treatment groups (Table 3).

DISCUSSION

Pentoxifylline is safe and well tolerated when given at the current dose of 1,200 mg. Prior uncontrolled pilot studies and more recent controlled pilot studies of PTX in NASH demonstrated improvement in liver enzymes, and weight loss with a dose of 1,600 mg/day.[10,13] We also demonstrated a significant correlation between weight loss and improvement in aminotransferases, which may explain one mechanism by which PTX could induce mild improvements in aminotransferases in patients with NASH. In addition, those patients who lost weight within the placebo group also demonstrated some level of improvement in aminotransferases.

In the present study, although PTX led to an improvement in serum aminotransferases as well as some histological features of NASH when compared to baseline measurements, there were no significant differences when compared to placebo. This finding underscores the importance of including a placebo (control) group in all NASH treatment studies.

Histologically, PTX had no effect on fibrosis; however, no drug to date has been able to consistently show an improvement in fibrosis in NASH clinical trials. The expression of genes integral to the development of fibrosis can herald changes before they are visible under the microscope and is less prone to sampling error. Although the histological fibrosis score did not decrease significantly in the PTX group, hepatic gene expression of collagen-1 was significantly reduced, and a trend of reduction was also seen in another important fibrogenic gene, TIMP-1. These data suggest that PTX may have a beneficial effect on fibrosis, although this requires further study.

The mechanism(s) by which PTX may impact liver injury remains to be fully defined. Others have shown that PTX has anti-TNF-α effects illustrated by a dose dependent reduction of TNF-α secretion in peripheral blood mononuclear cells of NASH patients after LPS stimulation.[8] However, our study did not show alterations in serum TNF-α. Moreover, we could not detect a statistically significant change in hepatic TNF-α gene expression, though this may have not been detected as a function of the small sample size. Similar to our findings, Akriviadis, *et al.* were not able to show a significant reduction in serum TNF-α levels in those treated with PTX compared to placebo for alcoholic hepatitis, a disease in which TNF-α has a well accepted role.[25]

Furthermore, in this alcoholic hepatitis trial, mortality improvement in the PTX group was directly related to a reduction in renal failure. The mechanism of action of PTX is complex and incompletely understood. In addition to its reported effect on TNF-α, another possible mechanism that may be beneficial to the liver is via improvement in microcirculatory blood flow as has been postulated in the kidney.[26,27]

CONCLUSION

While the present results are in line with prior pilot data suggesting PTX improves liver enzymes and possibly liver histology in NASH when making intra-group comparisons, when properly analyzed using inter-group comparisons, no statistically significant differences were found.

These data do demonstrate that PTX is a well-tolerated drug that may improve liver enzymes and histology in patients with NASH, but does not appear to offer substantial benefit over placebo. The most significant limitation of this study is the small sample size, thus the lack of statistical significance may be due to low power.

However, *a priori* calculations based on previously published literature indicated that a sample size of 30 patients was adequate to detect significant differences between groups. Furthermore, inclusion of patients with cirrhosis and those with normal aminotransferases at baseline may have underestimated the benefit of PTX. This data supports additional power calculations to pursue future larger phase IIb and III studies of PTX in NASH.

ABBREVIATIONS

- **NASH:** Non-alcoholic steatohepatitis.
- **TZDs:** Thiazoledinediones.
- **PTX:** Pentoxifylline.
- **cAMP:** Cyclic adenosine monophosphate.
- **TNFα:** Tumor necrosis factor alpha.
- **UPR:** Unfolded protein response.
- **NAFLD:** Non-alcoholic fatty liver disease.
- **NAS:** NAFLD activity score.
- **HOMA:** Homeostatic model assessment.
- **Ins_0:** Fasting insulin.
- **$Gluc_0$:** Fasting glucose.

- **MSD:** Multispot cytokine detection.
- **NASH CRN:** NASH clinical research network.
- **BMI:** Body mass index.
- **RT-PCR:** Real time quantitative polymerase chain reaction.

CONFLICT OF INTEREST

The authors have no conflicts of interest pertinent to this study.

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