

The Comparative Effects of Pentoxifylline and Ursodeoxycholic Acid on IL-1 β , IL-6, IL-8 AND TNF- α Levels in Nonalcoholic Fatty Liver

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Objective: To investigate the comparative effects of the pentoxifylline (PTX) and ursodeoxycholic acid (UDCA) on IL-1 β , IL-6, IL-8 and TNF- α in non-alcoholic fatty liver (NAFL) cases.

Method: Twenty-eight cases diagnosed to have NAFL were included in our study. The cases were divided into 3 groups. 20 mg/kg/day PTX was given to the subjects in group A (6 male, 4 female), 15 mg/kg/day UDCA was given to the subjects in group B (5 males, 5 females) for 6 months. The cases in group C (5 male, 3 female) were followed as control group. The biochemical values and cytokine levels of the cases were evaluated before and at the end of the sixth month of the treatment.

Results: When compared with the serum cytokine levels before and after the treatment, IL-8 and TNF- α levels were found to be significantly decreased both in group A and group B ($p < 0.05$), whereas there was no statistically significant change in IL-1 β and IL-6 levels ($p > 0.05$).

Conclusion: While PTX and UDCA significantly decreased the serum IL-8 and TNF- α levels in NAFL, their effects on IL-1 β and IL-6 were not significant.

Key words: Non-alcoholic fatty liver, pentoxifylline, ursodeoxycholic acid, cytokines

Non-alcoholic fatty liver (NAFL) has a clinically wide spectrum, which can progress ranging from a simple liver steatosis to steatohepatitis and cirrhosis (1). The disease shows the main histopathological characteristics observed in those patients with alcoholism, in spite of the fact that the patients do not use alcohol (2).

Pathogenesis of the disease is multifactorial. Some of the mechanisms responsible are oxidative stress, amino acid imbalance, hyperglycemia, imbalance between the ketogenic and antiketogenic hormones in the portal blood, endotoxemia and cytokine increase (2,3). Tumor necrosis factor alpha (TNF- α) and proinflammatory cytokines

induced by TNF- α (IL-8, IL-6, etc.) have key roles in liver diseases (4).

An effective treatment of NAFL is not available currently (4). Antibiotics and lactobacillus to decrease the endotoxemia originating from intestine, anti-TNF- α antibodies or receptor antagonists to inhibit the activity of TNF- α , glutathione derivatives, vitamin E, silymarin, propylthiouracil to decrease the free oxygen radicals; UDCA and gemfibrozil for cytoprotective effect and to decrease the hepatic lipids; leptin and thiozolidinedions to correct the insulin sensitivity and weight-lose are methods of treatment presently applied in animal and clinical studies (4, 5).

Pentoxifylline (PTX), which is a derivative of methylxanthine, is an immunomodulator agent affecting the cytokine formation. PTX is one of the potent inhibitors of both proinflammatory and antiinflammatory cytokines (6-8).

The aim of our study was to investigate the comparative effects of PTX, which is an inhibitor of TNF- α and UDCA on serum IL-1 β , IL-6, IL-8, TNF- α levels in NAFL cases.

Material and Method

Fourty-one cases having high serum aminotransferase, gamma-glutamyl transpeptidase (γ -GT) and/or alkaline phosphatase (AP) levels for more than three months together with hepatomegaly, steatosis in ultrasonography (US) or histopathological findings of NAFL in liver biopsy were included in this study.

In order to rule out other liver diseases, our cases were thoroughly evaluated with clinical examination and laboratory analyses: Individuals with any history of alcohol or drug use, a history of small intestinal resection, total parenteral nutrition, diabetes mellitus, known liver diseases and malignancies were not included in the study. Before the treatment; age, gender, height, weight, body mass index (BMI) of the cases were recorded. BMI was calculated with weight kg/height² (m²) formula. BMI values were considered to suggest following conditions (male/female):

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Table I. Demographic and clinic characteristics of subjects

Parameters	Group A (PTX)	Group B (UDCA)	Group C (control)
	n=10	n=10	n=8
Mean age (years)	47.2±8.7	50.2±1	46.8±4
Male/Female	6/4	5/5	5/3
Malaise	4 (40%)	7 (70%)	4 (50%)
Abdominal pain	4 (40%)	3 (30%)	2 (25%)
Obesity	3 (30%)	4 (40%)	1 (12.5%)
Hepatomegaly	5 (50%)	6 (60%)	3 (37.5%)
Body Mass Index	29±2	27.9±2	26.8±3
Hypercholesterolemia	4 (40%)	6 (60%)	4 (20%)
Hypertriglyceridemia	5 (50%)	7 (70%)	3 (20%)
Liver biopsy	8 (80%)	6 (60%)	2 (25%)

Table II. Biochemical values of group A and B in pre- and post-treatment

Parameters	Group A (PTX)			Group B (UDCA)		
	Pretreatment	Posttreatment	p	Pretreatment	Posttreatment	p
Glucose (mg/dl)	82.4±9.8	78±6.5	NS	80.2±10	78.5±9.3	NS
AST (U/L)	44.6±8.7	24.9±6.3	<0.05	68.2±32	30.1±5.9	<0.05
ALT (U/L)	81.2±20.5	28.2±6.3	<0.05	119.6±64	37±10.6	<0.05
AST/ALT	0.5	0.9		0.6	0.8	
AP (U/L)	191.6±60	142.6± 52	NS	207±73	178±49	NS
GGT (U/L)	85.7±38	30.9± 8	<0.05	87.2±22	38.4±14	<0.05
Cholesterol (mg/dl)	183.7±52	185.2±34	NS	216.5±48	201.6±42	NS
Triglyceride (mg/dl)	259.4±90	182.4±70	<0.05	265.5±149	236.7±98	NS

Table III. Comparison of the groups pre- and post-treatment cytokine levels

	Group A (PTX)			Group B (UDCA)			Group C (Control)		
	Before	After	p	Before	After	p	Before	After	p
IL-1β pg/ml	2.5± 4.1	2.1± 2.2	0.87	3.0± 3.9	3.0± 3.0	0.57	2.0± 2.3	2.2± 1.8	0.77
IL-6 pg/ml	4.0± 3.2	3.5 ± 2.3	0.07	4.0± 2.2	4.1± 2.6	0.83	4.4± 4.4	4.7 ± 3.0	0.26
IL-8 pg/ml	36.9±34.4	7.8± 6.4	0.007	30.2±42.7	5.2± 5.0	0.009	34.9± 18.4	35± 23.8	0.88
TNF-α pg/ml	7.5± 5.1	4.2 ± 2.2	0.04	6.3± 3.6	4.6± 1.7	0.03	6.7 ± 5.1	6.6±4.1	0.48

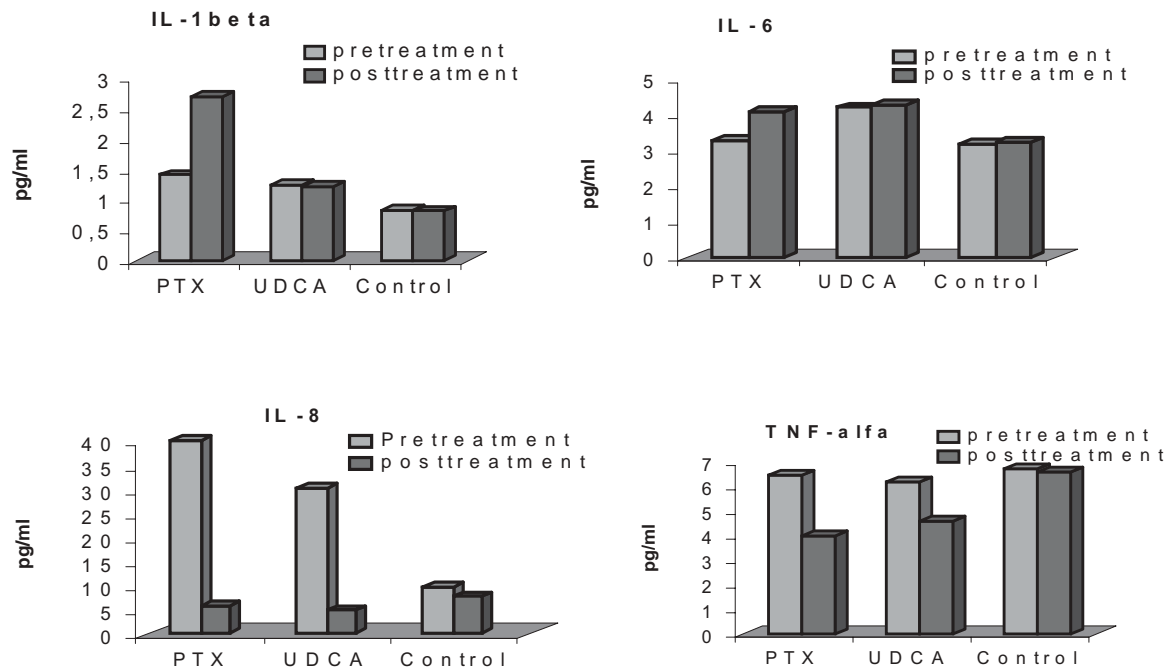


Figure 1. The effects of the PTX and UDCA on the serum cytokine levels

Normal 18,5-23.5/19.5-24.5 (both sexes); overweight: 23.5 -29.5; obese:>29.5.

Laboratory evaluation included serum aspartate transaminase (AST), alanine transaminase (ALT), γ -GT, AP, cholesterol, triglyceride, fasting glucose, auto-antibodies (antinuclear antibody, antismooth muscle antibody and antimitochondrial antibody), iron profile, ceruloplasmin and α 1-antitrypsin levels. Hepatitis B serology and antibody to hepatitis C virus were investigated and complete blood count was conducted. Cases were evaluated with their IL-1 β , IL-6, IL-8, TNF- α levels before and at the end of the sixth month of the treatment. US examination of all cases and liver biopsy of those who gave written consent were performed before the treatment. Biopsy specimens taken with Menghini needle aspiration technique were evaluated by the same pathologist and the presence of liver steatosis in specimens was investigated histopathologically.

The cases were divided into 3 groups. The patients in group A (11 males, 6 females) were given 20 mg/kg/day PTX (Trental, Hoechst Marion Roussel, Germany); in group B (9 males, 7 females) 15/mg/kg day UDCA (Ursofalk, Dr. Falk GmbH and Co, Germany) for 6 months. Cases in group C (5 males, 3 females) were followed as the control group. No special diet was given to the subjects during the treatment period.

All of the serum samples were preserved at -70 °C. Serum cytokine levels were measured by using Immulite IL-1 β , IL-6, IL-8 and TNF- α commercial kits (Bio DPC, Los Angeles, USA) with chemiluminescent immunometric assay with Immulite hormone analyser.

The results were calculated as mean \pm standard error

(SEM). For statistical comparison of the three groups with each other before and after the treatment variance analysis (One-way ANOVA), for comparison within the groups themselves variant analysis and Wilcoxon tests were used. A p value <0.05 was considered significant.

Results

Ten cases in group A (6 males, 4 females; mean age: 47.2 \pm 8.7) and 10 cases in group B (5 males, 5 females; mean age: 50.2 \pm 10) completed the treatment and only the data obtained from these cases were used for analyses. Thirteen cases (7 in group A, 6 in group B) were excluded from the study: nine of them did not use their drugs properly and the others did not attend follow-up. All cases in group C (5 males, 3 females; mean age: 46.8 \pm 4) completed follow-up period.

Before the treatment, there was no difference in terms of age, gender and characteristics of clinic between the groups. In group A; all of the cases, three of whom were obese, were considered to be overweighted, in group B; except one case, all were overweighted, obesity was determined in 4 cases in this group, in group C; all of the cases were overweighted, obesity was detected in only one case. Hepatomegaly was present in five cases in group A, six cases in group B and three cases in group C. The demographic features of the cases in our study are shown in Table I.

Liver biopsy was performed in 16 of the cases (8 from group A, 6 from group B and 2 from group C). Of the cases that biopsy was performed on, 11 were determined to have macrovesicular steatosis, three cases were observed to have mixed type and two cases were detected to have

microvesicular steatosis. Intralobular inflammation was present in two of the cases with macrovesicular steatosis. Twelve cases that biopsy specimens could not be taken from were included in our study after they were excluded from other probable liver diseases depending laboratory and US findings.

Serum ALT levels were abnormal in all of the patients at the beginning of the study. Serum cytokine levels prior to the treatment were determined as follows: in group A; IL-1 β in two of the cases, IL-6 in one, IL-8 in three, TNF- α in three, in group B; IL-1 β in two of the cases, IL-8 in two, TNF- α in five, in group C; IL-1 β in one of the cases, IL-6 in two, IL-8 in one and TNF- α in three of the cases; these values were determined to be over the cut-off values. When three groups were compared in terms of the mean cytokine levels prior to the treatment, no significant differences were detected statistically among the groups.

No significant difference was determined statistically between BMIs of the groups before and after the treatment. Side effects in the four patients in the PTX treatment group included headache, abdominal pain, nausea and itching. In three patients in UDCA treatment group, side effects included mild diarrhea and abdominal pain.

In both PTX and UDCA treatment groups ALT, γ -GT, AST levels significantly decreased at the end of the treatment (Table II), whereas in the control group no significant difference was detected. After the treatment serum cytokine levels in group A; IL-1 β in one of the cases, in group B; IL-1 β in two of the cases, in group C; IL-1 β in one of the cases, IL-6 in one, IL-8 in two and TNF- α in three were determined over the cut-off values. When three groups were compared in terms of the mean cytokine levels after the treatment, only IL-8 levels between group A-group C and group B-group C were determined to be significantly different ($p < 0.05$).

In the evaluation of the groups in terms of serum cytokine levels before and after the treatment, IL-8 and TNF- α levels were found to be significantly decreased both in group A and group B ($p < 0.01$ and $p < 0.05$), IL-1 β and IL-6 levels were not found to be significantly different in all of the groups ($p > 0.05$), whereas no statistically significant change was observed in cytokine levels in individuals of the group C before and after the treatment (Table III) (Figure 1).

Discussion

Although NAFL is usually benign, in some cases it may progress to steatohepatitis, fibrosis and cirrhosis (10,11). Many factors are believed to play role in its etiology. Obesity is the major risk factor. Rapid weight loss, diabetes, total parenteral nutrition, jejunio-ileal bypass, Wilson disease, hepatitis C infection, drugs and toxins are most frequently determined causes (11,12)

There exist no accepted treatment for the disease. At present, too many pharmacological agents are investigated for the treatment of NAFL. Since many of the patients are obese, dietary therapy is the first step in the treatment

(4,11,13). Weight loss may decrease the concentration of serum aminotransferase in hepatosteatosis. However, fast and excessive weight loss may accelerate the progression from steatosis to the cirrhosis. For this reason, decreasing the weight is not always an effective treatment, sometimes it can increase the severity of the disease (4). It's known that Vitamin E, silymarin, gemfibrozil, betaine, N-acetylcysteine and some other agents such as UDCA are useful for they decrease lipid peroxidation through their antioxidant effect (1,14)

In our study, we investigated the effects of PTX and UDCA on some cytokines (IL-1 β , IL-6, IL-8, TNF- α) in NAFL cases. At the end of sixth month no changes were observed in BMIs of groups when compared with those before the treatment. Both in PTX and UDCA groups at the end of the sixth month, while statistically significant decreases were determined in serum mean IL-8 and TNF- α levels after the treatment, no changes were observed in the levels of control group before and after the treatment. This decrease was more significant especially in IL-8. PTX and UDCA have similar effects on IL-8 and TNF- α levels and there was no statistically significant difference between them. No important effects of either drugs on pre- and post-treatment IL-1 β and IL-6 levels were determined (Table III).

Cytokines are pleiotrophic regulatory peptides synthesized by nucleated cells. Many liver cells such as Kupffer cells, hepatocytes, stellate cells can synthesize cytokines. Viruses, alcohol and toxins in early stages of chronic liver disease and endotoxins in late stage of the disease play a crucial role in stimulating cytokine production. There are many cytokines that play role in liver diseases (4). Cytokines cause necroinflammation in liver by increasing the accumulation of free fatty acids and inactivating cytochrome P450 (15).

TNF- α synthesized by macrophages and monocytes is a potent inhibitor of lipoprotein lipase. Kupffer cells in the liver are the major source of TNF- α . Increased TNF- α by inhibiting lipolysis in the peripheral tissues increases synthesis and accumulation of triglyceride in liver (2,4,17). It's found that increased lipopolisaccharides in blood resulted from endotoxemia cause hepatosteatosis by inducing TNF- α in obese rats and this steatosis is more significant in females than in males (18). There are studies with controversial results in the literature. Memon et al. (19) showed that TNF- α is not the cause of fatty liver disease in obese diabetic rats.

IL-8 is a cytokine synthesized by hepatocytes, Kupffer cells and macrophages. It shows its effects by activating neutrophils. Serum level of IL-8 was increased significantly in alcoholic and non-alcoholic hepatosteatosis (20).

PTX is a methylxanthine derivative having hemorrhagic, hemodynamic and anti-inflammatory effects, which is used in the treatment of several vascular insufficiencies in clinics for a long time. In experimental studies it was found that PTX decreases serum TNF- α

level and portal blood flow (7). There are different reports from independent studies related with the effects of PTX on cytokines. It was showed that PTX suppresses the formation of TNF- α and IL-1 β , IL-2, IL-10 (6, 7, 21). Similarly Weinberg et al. (22) found that PTX suppresses the expression of IL-1 β and TNF- α in leukaemia cells. However, Vromen et al. (23) showed that PTX has no roles in preventing endotoxemia caused by total parenteral nutrition and hepatosteatosis due to TNF- α . In our study, we determined that PTX decreases mean serum level of IL-8 and TNF- α in NAFL cases at the end of six month treatment, but had no effect on IL-1 β and IL-6 levels.

Ursodeoxycholic acid may be useful in NAFL patients. It inhibits intestinal absorption and secretion of cholesterol and formation of cholesterol crystals in bile. It provides cytoprotective effect by protecting the lipid layer of hepatic cell membranes against toxic effect of hydrophobic bile salts (24). In a study, it was showed that it corrects both steatosis and enzymes, but does not affect fibrosis and inflammation of liver (3). Laurin et al. (12) determined significant improvement in levels of ALP, ALT, γ -GT and liver steatosis in nonalcoholic hepatosteatosis patients whom they had applied 12 months UDCA treatment.

There are a few studies on the effect of UDCA on serum cytokine levels. Neuman et al. (25) demonstrated in vitro that, UDCA decreases the cytotoxicity in Hep G2 cells formed by ethanol, suppresses release and expression of IL-1 α , IL-6 and TNF- α . In another study, it was showed that UDCA decreases the translocation of lipopolysaccharides due to endotoxemia and prevents the cytokine response (26). In our study, similar results were found. We determined that UDCA decreased the post-treatment mean serum levels of IL-8 and TNF- α significantly, but did not affect IL-1 β and IL-6 levels.

Recently, Lin et al (27) showed that metformin normalizes aminotransferases by inhibiting hepatic expression of TNF- α , which improves steatosis in hepatosteatosis cases.

When we consider important roles of the proinflammatory cytokines in pathogenesis of NAFL, therapeutical approaches in the future will be directed to prevention of the release at these factors. With this purpose; the clinical use of agents to prevent endotoxemia (antibiotic, lactobacillus), anti-cytokine antibodies or receptor antagonists, antioxidant and lipid lowering agents will make the treatment of liver disease or NAFL possible.

As a result; PTX and UDCA were found to be effective in decreasing the serum IL-8 and TNF- α levels playing roles in pathogenesis of NAFL. However, randomised, controlled and long-lasting studies on the use of each drug in NAFL are needed.

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