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A comparison of the effects of infliximab, adalimumab, and pentoxifylline on rats with non-alcoholic steatohepatitis

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Original Article

ABSTRACT

Background/Aims: Non-alcoholic steatohepatitis (NASH) lacks effective medical treatment.Since tumor necrosis factor alpha (TNF-α) plays an important role in NASH pathogenesis, we aimed to investigate drugs affecting TNF-α as possible treatment options during the development of NASH.

Materials and Methods: A total of 35 rats were divided into five groupsand evaluated over a 6week period. One group received a normal diet alone or in combination with the administration of infliximab, adalimumab or pentoxifylline

Results: NASH was successfully established in the MCD diet group. Levels of TNF-α were effectively suppressed in the three groups that received anti-TNF agents. No statistically significant differences were observed between the three agents in terms of the histological NASH score.

Conclusion: Our study showed that the anti-TNF agents infliximab, adalimumab, and pentoxifylline effectively suppress TNF-α. Although these drugs did not prevent the development of NASH, they were able to slightly reverse the NASH histopathology score and positively affect liver function tests

Keywords: Non-alcoholic steatohepatitis, infliximab, adalimumab, pentoxifylline, rats.

INTRODUCTION

The concept of non-alcoholic steatohepatitis (NASH) was first described by Ludwig and colleagues in 1980 (1). Non-alcoholic fatty liver diseases encompass a wide spectrum of liver damage ranging from NASH to fibrosis, cirrhosis, and steatosis. When only NASH is developed during the natural course of disease, patient have a propensity to develop fibrosis and cirrhosis as well. The pathogenesis of NASH is very complex. The damage begins with insulin resistance. Further damage to the liver is necessary for the continuation of steatohepatitis. The interaction between oxidative stress and cytokines has been suggested to play a key role in NASH. The increased production of TNF-α was observed in NASH rat models, and increased serum TNF-α concentrations were also reported in humans with NASH (2,3,4).

A methionine- and choline-deficient (MCD) diet impairs the synthesis of phosphatidylinositol choline, which is required for synthesis of very-low-density lipoprotein. In phosphatidylinositol choline deficiency, serum triglyceride levels are decreased while liver triglyceride levels are increased. As a result, steatosis is increased (5,6). An MCD diet displaces hepatic oxidants such as glutathione and S-adenosylmethionine (7). An MCD diet increases oxidative stress by suppressing oxidative defense mechanisms (8), which induces TNF-α and other proinflammatory cytokines (9). In addition, an MCD diet leads to non-alcoholic fatty liver and insulin resistance via similar mechanisms. Therefore, MCD model studies are very elucidative because they emphasize the importance of oxidative stress in the pathogenesis of hepatic steatosis and steatohepatitis (independent of obesity). Aminotransferase levels, histological changes in the liver characterized by steatosis, focal inflammation, hepatocyte necrosis, and fibrosis are induced in mice fed the MCD diet. These rapid histological changes in morphology are similar to those seen in human patients with NASH (10,11).

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Currently, there is no ideal treatment for NASH. Lipid-lowering agents such as statins and fibrates (12, 13), antioxidants such as vitamin E (14,15,16), and cytoprotective agents such as ursodeoxycholic acid have been shown to reduce the level of aminotransferase. In addition, they were shown to improve liver histology in several studies (17). However, none of these drugs are specific for or target NASH. Therefore, there is a need for specific drugs targeted for the treatment of NASH.

Infliximab, which is a TNF-α antibody, is widely used in the treatment of various diseases such as rheumatoid arthritis and inflammatory bowel disease (18,19). Two studies have shown that infliximab reduced liver damage in NASH rats (20,21).

Adalimumab is a humanized antibody against TNF-αused to treat rheumatoid arthritis and Crohn's disease. A permanent response in biochemical parameters was seen in a NASH patient who was administered adalimumab as a rheumatoid arthritis treatment (22). However, there have been no published studies on the use of adalimumab to treat NASH.

Pentoxifylline is a non-selective phosphodiesterase inhibitor that decreases TNF-α gene transcription and also inhibits multiple steps of cytokine/chemokine pathway, thus inhibiting TNF-α directly or indirectly. In rat models and human studies, pentoxifylline has been shown to improve transaminase levels and liver histopathology (23,24,25,26).

Since TNF-α plays an important role in the pathogenesis of NASH, we aimed to investigate the effects of the anti TNF-α agents infliximab, adalimumab, and pentoxifylline NASH pathogenesis in rats.

MATERIALS AND METHODS

Animals:

A total of 35 male Wistar albino rats weighing 242-351 g (mean298.7 g) were obtained from the Experimental Research Laboratory, Faculty of Medicine, Dokuz Eylül University Hospital. Rats were put into cages and maintained according to the international animal experimentation and maintenance manual under standard laboratory conditions (temperature 23±2 °C, 12-hour day/night cycle).

Diet:

Rats were given either a normal diet or an MCD diet, the content and chemical structure of which was standardized by animal diet experts (Harlan Teklad, Madison, WI, U.S.). The rats were given 10g food/100 g body weight and 10-12 ml water/100 g body weight daily.

Study design:

Thirty-five male Wistar albino rats were divided into five equal groups of 7 rats each (Figure 1).

Group 1 (n=7, control): Rats in this control group were fed with standard food and water.

Group 2 (n=7, MCD): Rats from this group received an MCD diet for 6 weeks.

Group 3 (n=7, MCD+infliximab): Rats in this group were administered 4 mg/kg infliximab (infliximab dose adjusted for rats) intraperitoneally at weeks 0 and 2 and were fed an MCD diet and water for 6 weeks.

Group 4 (n=7, MCD+adalimumab): Rats in this group received 7mg/kg adalimumab intraperitoneally at weeks 0, 2, and 4 and were fed an MCD diet and water for 6 weeks. Although no studies have reported using adalimumab in NASH rat models, in vitro models and other studies with rats suggested that the dose of 7mg/kg was safe.

Group 5 (n=7, MCD+pentoxifylline): The rats in this group were administered pentoxifylline at 4.5mg/kg/day and were fed an MCD diet and water for 6 weeks. During week 4 of the study one of the rats in this group killed another, and therefore the study was completed with only 6 rats.

Termination of the experiment and collecting samples

Six weeks after initiation of the study, the abdomen was opened under ether anesthesia and approximately 10 cc of blood was drawn from the vena cava. Blood samples were centrifuged at 5000 rpm for 5 minutes and stored at -80 °C analysis were made. Liver tissues were placed in 10% formalin and sent to the pathology laboratory for histopathological examinations. Rats were sacrificed by ether inhalation.

Cytokine levels

TNF-α, TGF-β, IL-6 (eBioscience Int, San Diego, CA, USA), and IL-8 (Cusabio Int, Wuhan, Hubei, PRC) levels were measured by ELISA.

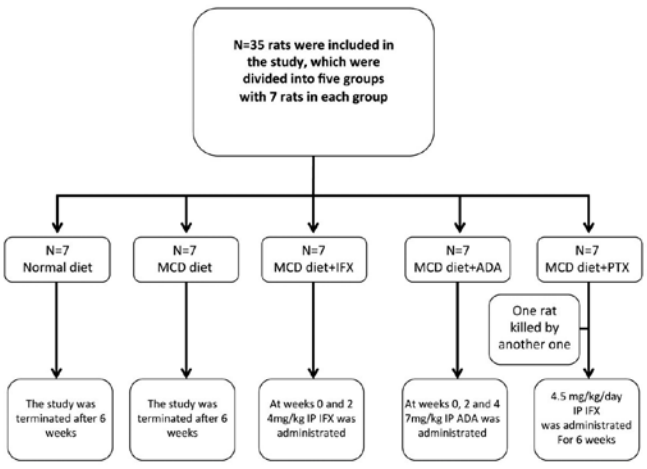

Figure 1. Groups and their characteristics.
MCD: Methionine- and choline-deficiet, IFX: infliximab, ADA: adalimumab, PTX: pentoxifylline, IP: intraperitoneal

Biochemical analysis

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, total protein, and albumin levels were assessed using an Abbott Architect 16000 autoanalyzer (Abbott Inc., Princeton, NJ, USA).

Histopathological evaluation

Liver tissue samples were obtained and fixed in paraffin blocks for processing using traditional methods for histological examination. The liver was cut for hematoxylin-eosin staining. The prevalence of fibrosis was determined Masson trichrome staining. An expert pathologist examined the samples under a light microscope. The presence or absence of steatosis, ballooning degeneration, lobular inflammation, and fibrosis was assessed, and Brunt NASH scoring was performed. Scores of five and above were considered diagnostic for NASH.

Statistics

SPSS 15.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Differences between the groups were determined by Kruskal-Wallis and Mann-Whitney U tests, and p values <0.05 were considered statistically significant.

RESULTS

All of the rats from each of the 5 groups were weighed once a week starting with the first day. A distinct weight gain was observed in rats that were fed a normal diet. Rats in the four groups fed with the MCD diet lost weight. Their weight loss was more distinct during the first 3 weeks and decreased during the last 3 weeks. The weekly average weight for each of the groups is shown in Figure 2. No side effects were observed throughout the study. In the group receiving the MCD diet and pentoxifylline, one of the rats attacked and killed another, and therefore this group included 6 rats at study completion.

Immunological parameters:

The average serum TNF-α, TGF-β, IL6, and IL8 levels are shown in Table 1 and Figure 3. Statistically different parameters between the groups were determined by the Kruskal-Wallis test. The statistically significant parameters between the groups were identified by the Mann-Whitney U test (Table 2).

The average TNF-α level 21.57±5.89 pg/mL in the control group, 32.37±13.85 pg/mL in the MCD group, 22.86±16.15 pg/mL in the MCD+infliximab group, 11.49±8.10 pg/mL in the MCD+adalimumab group, and 29.67±5.65 pg/mL in the MCD+pentoxifylline group. TNF-α levels were thus highest in

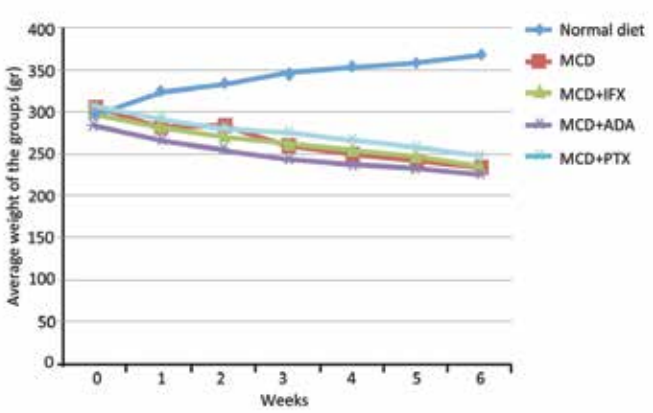

Figure 2. Weekly awerage weight of the groups.
MCD: Methionine- and choline-deficient, IFX: infliximab, ADA: adalimumab, PTX: pentoxifylline

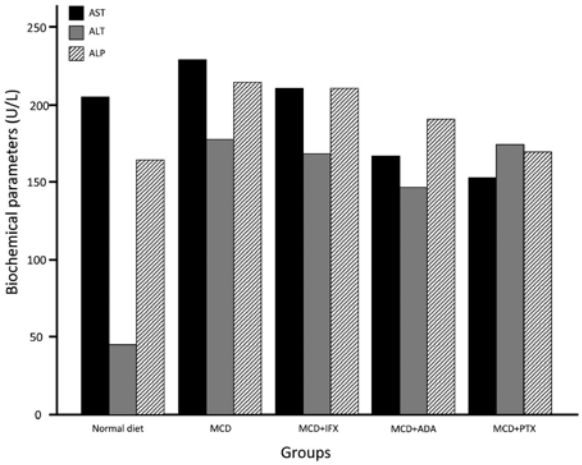

Figure 3. TNF-α, TGF-β, IL6, and IL8 levels.
MCD: Methionine- and choline-deficient, IFX: infliximab, ADA: adalimumab, PTX: pentoxifylline

Table 1. TNF-α, TGF-β, IL6, and IL8 levels

Immunological parameters	All rats (n=34)	Normal diet (n=7)	MCD (n=7)	MCD+IFX (n=7)	MCD+ADA (n=7)	MCD+PTX (n=6)	p*
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
TNF-α (pg/mL)	21.65(12.35)	21.57(5.89)	32.37(13.85)	22.86(16.15)	11.49(8.10)	19.67(5.65)	0.005
TGF-β (pg/mL)	47.58(17.08)	54.56(11.92)	69.48(11.47)	47.53(10.36)	34.99(6.77)	28.67(4.90)	<0.001
IL6 (pg/mL)	173.67(64.92)	202.45(28.72)	181.39(8.44)	133.83(34.54)	96.51(41.13)	267.58(29.95)	<0.001
IL8 (pg/mL)	2.05(1.18)	1.65(0.93)	2.81(1.87)	1.28(0.35)	2.10(0.98)	2.49(0.71)	0.014

*Kruskal-Wallis test
MCD:Methionine-and choline-deficient
IFX: infliximab
ADA: adalimumab
PTX: pentoxifylline

Table 2. Statistical comparison of cytokine levels between groups

	TNF -α		TGF-β		IL6		IL8	
Groups	**Z**	**p***	**Z**	**p***	**Z**	**p***	**Z**	**p***
Normal diet vs MCD	-2.236	0.025	-1.981	0.048	-.958	0.338	-1.853	0.064
MCD vs MCD+IFX	-1.981	0.048	-2.747	0.006	-2.492	0.013	-2.619	0.009
MCD vs MCD+ADA	-2.619	0.009	-3.130	0.002	-3.130	0.002	-.958	0.338
MCD vs MCD+PTX	-2.286	0.022	-3.000	0.003	-3.000	0.003	-.143	0.886
MCD+IFX vs MCD+ADA	-2.236	0.025	-2.108	0.035	-1.469	0.142	-2.047	0.041
MCD+IFX vs MCD+PTX	-.286	0.775	-3.000	0.003	-3.000	0.003	-2.857	0.004
MCD+ADA vs MCD+PTX	-2.286	0.022	-1.714	0.086	-3.000	0.003	-1.216	0.224

* Mann-Whitney U test
MCD:Methionine- and choline-deficient
IFX: infliximab
ADA: adalimumab
PTX: pentoxifylline

Table 3. Biochemical parameters

Biochemical parameter	**All rats (n=34)** Mean (SD)	**Normal diet (n=7)** Mean (SD)	**MCD (n=7)** Mean (SD)	**MCD+IFX (n=7)** Mean (SD)	**MCD+ADA (n=7)** Mean (SD)	**MCD+PTX (n=6)** Mean (SD)	**p***
AST (U/L)	194.05 (53.80)	205.57 (11.73)	228.57 (67.37)	210.71 (65.68)	166.85 (35.03)	152.66 (38.90)	0.043
ALT (U/L)	141.35 (76.86)	45.00 (7.28)	177.71 (77.25)	168.14 (71.66)	146.42 (53.86)	174.16 (70.28)	0.002
ALP (U/L)	190.50 (59.85)	164.14 (49.34)	214.71 (72.46)	210.71 (54.15)	190.42 (71.43)	169.50 (43.44)	0.473
Bilirubin (mg/dl)	0.19 (0.07)	0.11 (0.01)	0.19 (0.06)	0.23 (0.06)	0.20 (0.10)	0.23 (0.06)	0.008
Total protein (g/dl)	6.47 (1.19)	7.10 (0.54)	6.15 (0.35)	6.58 (0.81)	6.54 (2.39)	5.88 (0.21)	0.002
Albumin (g/dl)	3.08 (0.37)	3.25 (0.05)	3.148 (0.28)	3.02 (0.35)	3.02 (0.66)	2.96 (0.23)	0.073

*Kruskal-Wallis test
MCD:Methionine- and-choline deficient
IFX: infliximab
ADA: adalimumab
PTX: pentoxifylline
ALT: alanine aminotransferase
AST: aspartate aminotransferase
ALP: alkaline phosphatase

the MCD group and lowest were in the MCD+adalimumab group.

Biochemical parameters:

The mean serum AST, ALT, ALP, bilirubin, total protein, and albumin levels in all groups are shown in Table 3 and Figure 4 and 5. Statistically different parameters between the groups were determined with the Kruskal-Wallis test. The parameters that were identified to be statistically significant were evaluated by a Mann-Whitney U test (Table 4).

Histopathology:

The liver steatosis, lobular inflammation, hepatocyte ballooning, fibrosis, and total NASH scores for all groups are shown in Table 5. Parameters that significantly differed between the groups were determined with the Kruskal-Wallis test. Since the NASH score was zero in the group fed a normal diet, this group was excluded from the Kruskal-Wallis test. The Mann-Whitney U test detected a statistically significant difference between the groups in terms of hepatocyte ballooning (Table 6). As expected, histologically examination confirmed that NASH had developed in the group fed the MCD diet. Rats with a total score of 5 or higher were considered to demonstrate NASH.

When the total NASH score was compared between groups, the highest total NASH score of6.14±1.06 was observed in the MCD group, while the lowest score, 5.28±0.75, was observed in the MCD+ adalimumab group. However, there were no significant differences between groups. The only significant difference in histopathological findings was observed in hepatocyte ballooning, in which the MCD group significantly differed the MCD+infliximab group (p=0.044) and the MCD+adalimumab group (p=0.018). The difference between the MCD group and the MCD+ pentoxifylline group was not statistically significant,

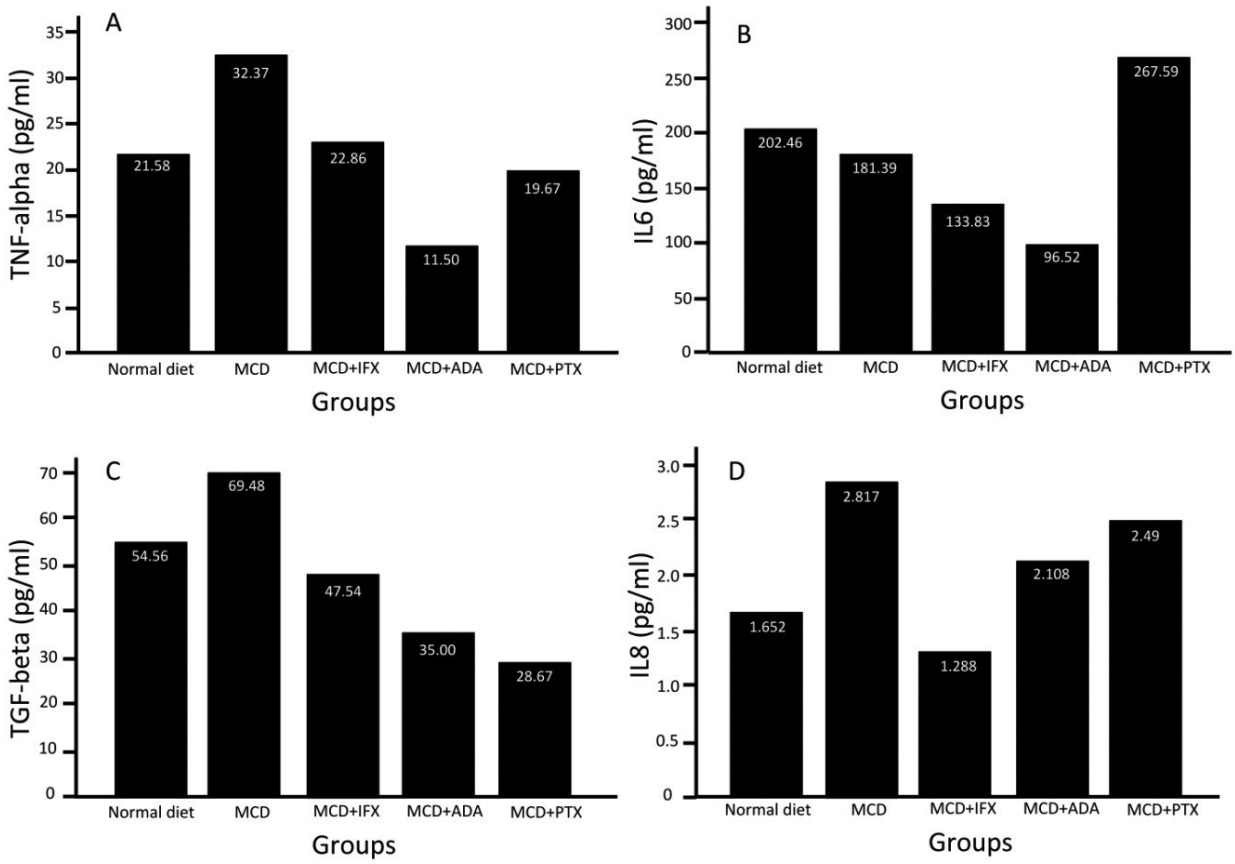

Figure 4. AST, ALT and ALP levels.
MCD: Methionine- and choline-deficient, IFX: infliximab, ADA: adalimumab, PTX: pentoxifylline

Table 4. Statistical comparison of biochemical parameters between groups

	AST		ALT		Bilirubin		T. protein	
Group	**Z**	**p***	**Z**	**p***	**Z**	**p***	**Z**	**p***
Normal diet vs MCD	-0.44	0.654	-3.13	0.002	-2.97	0.003	-2.82	0.005
MCD vs MCD+IFX	-0.44	0.654	-0.06	0.949	-1.35	0.177	-.904	0.366
MCD vs MCD+ADA	-1.59	0.110	-0.70	0.482	-0.19	0.848	-1.41	0.158
MCD vs MCD+PTX	-1.85	0.063	-0.14	0.886	-1.07	0.281	-1.44	0.150
MCD+IFX vs MCD+ADA	-1.47	0.141	-0.44	0.655	-0.57	0.565	-2.05	0.040
MCD+IFX vs MCD+PTX	-1.86	0.063	-0.14	0.886	-0.07	0.943	-2.66	0.008
MCD+ADA vs MCD+PTX	-0.57	0.568	-1.00	0.317	-0.71	0.474	-0.43	0.666

* Mann-Whitney U test
MCD:Methionine- and choline-deficient
IFX: infliximab
ADA: adalimumab
PTX: pentoxifylline

although the group given pentoxifylline demonstrated a trend toward lower hepatocyte ballooning scores (p=0.053). The mean NASH scores of all groups are shown in Figure 6, and the histopathological results are shown in Figure 7.

DISCUSSION

This study successfully established a NASH model using an MCD diet. At the end of 6weeks, we found that serum TNF-α levels were statistically higher in the group fed an MCD diet coparated to the control group fed a normal diet, as were TGF-β levels indicating that the group with an established NASH model had increased levels of both these cytokines. IL6 levels did not increase in our NASH model. IL8 levels were higher in the MCD group than in the control group; however this increase was not statistically significant. Comparison of biochemical parameters, indicated AST, ALT, and ALP levels were also increased in mice

Table 5. Histopathological scores

Histopathological scores	All rats (n=34) Mean (SD)	Normal Diet (n=7) Mean (SD)	MCD (n=7) Mean (SD)	MCD+IFX (n=7) Mean (SD)	MCD+ADA (n=7) Mean (SD)	MCD+PTX (n=6) Mean (SD)	p**
Steatosis	2.38 (1.23)	0 (0)	3.00 (0)	3.00 (0)	3.00 (0)	3.00 (0)	1.000
Lobular Inflammation	1.50 (0.99)	0 (0)	2.14 (0.89)	2.00 (0.81)	1.71 (0.48)	1.66 (0.51)	0.605
Hepatocyte ballooning	0.73 (0.61)	0 (0)	1.42 (0.53)	0.85 (0.37)	0.57 (0.53)	0.83(0.40)	0.032
Fibrosis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.000
Total NASH Score	4.52 (2.47)	0 (0)	6.14 (1.06)	5.85 (1.06)	5.28 (0.75)	5.50 (0.54)	0.357

** Kruskal-Wallis test (the normal diet group was excluded)
MCD:Methionine- and choline-deficient
IFX: infliximab
ADA: adalimumab
PTX: pentoxifylline

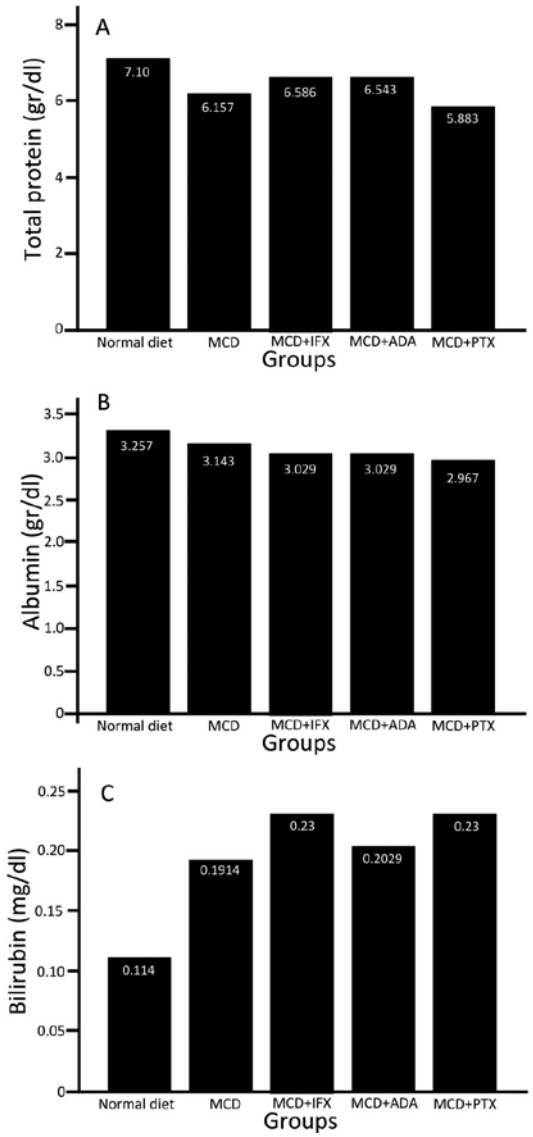

Figure 5. Total protein, albumin and total bilirubin levels.
MCD: Methionine- and choline-deficient, IFX: infliximab, ADA: adalimumab, PTX: pentoxifylline

Table 6. Statistical comparison of hepatocyte ballooning between groups

Groups	Hepatocyte ballooning Z	p*
MCD vs. MCD+IFX	-2.01	0.044
MCD vs. MCD+ADA	-2.36	0.018
MCD vs. MCD+PTX	-1.93	0.053
MCD+IFX vs. MCD+ADA	-1.14	0.254
MCD+IFX vs. MCD+PTX	-0.11	0.909
MCD+ADA vs. MCD+PTX	-0.98	0.327

* Mann-Whitney U test
MCD: Methionine- and choline-deficient
IFX: infliximab
ADA: adalimumab
PTX: pentoxifylline

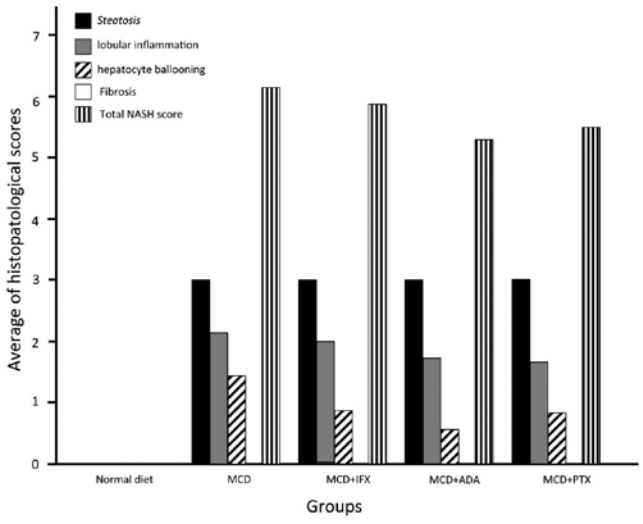

Figure 6. NASH scores.
MCD: Methionine- and choline-deficient, IFX: infliximab, ADA: adalimumab, PTX: pentoxifylline

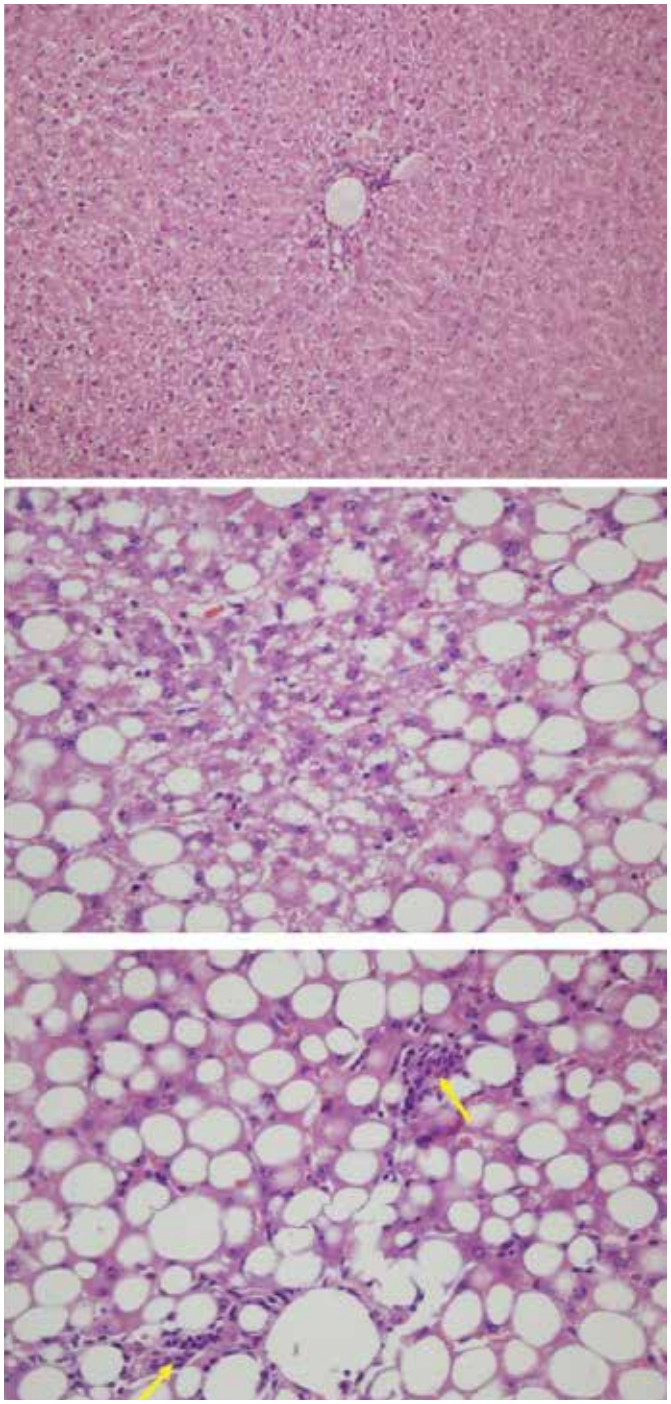

Figure 7. The histological appearance of the liver from a rat fed a normal diet (20x magnification, H&E) **(a)**. Hepatocyte ballooning in the group fed a Methionine- and choline-deficient (MCD) diet (20x, H&E) **(b)**. Steatosis and lobular inflammation in hepatocytes of rats fed an MCD diet (20x, H&E, arrows indicate lobular inflammation) **(c)**.

fed an MCD diet compared to the group that received a normal diet. However, the only significant increase was in ALT levels. In addition, total bilirubin levels were also significantly higher in the MCD-fed groups than in the control group.

On histopathological evaluation, the control group had a NASH score of zero. The establishment of NASH was histopathologically confirmed the groups fed an MCD diet. The development of fibrosis was not observed in mice with established NASH. In combination with our finding, consistent with previous studies, that the MCD diet increased pro-inflammatory cytokines (except IL 6) involved in the pathogenesis of NASH, these findings indicated that our NASH model had been successfully established.

Barbuio and colleagues established a rat NASH model using a high-fat diet and demonstrated that infliximab significantly reduced the expression of pro-inflammatory markers and reversed fibrosis and fat accumulation by correcting insulin signal transduction in those rats (27).

Koca et al. determined that infliximab decreased AST, ALT, TNF-α, and TGF-β1 levels and also reversed steatosis, inflammation, and fibrosis when compared to a placebo in MCD diet-induced NASH (28).

In our study, the MCD+infliximab group demonstrated significant suppression of serum TNF-α levels compared to the group that received only an MCD diet. Instead, TNF-α levels in the group that received infliximab were similar to those of the control group, which was fed a normal diet and did not develop NASH. This result indicates that infliximab plays a role in the suppression of TNF-α. Similarly, IL6, IL8, and TGF-β levels were significantly suppressed in the group that received infliximab. The steatosis score of as both the MCD and MCD+infliximab groups was 3, and the lobular inflammation score was also similar between group those groups. However, hepatocyte ballooning was significantly reduced in the MCD+infliximab group. The total NASH score of the MCD+infliximab group was lower than that of the MCD group, but this difference was not significant. The tested biochemical parameters (AST, ALT, ALP, bilirubin, albumin, and total protein) did not significantly differ between the MCD and the MCD+infliximab. However, slight decreases were observed in AST, ALT, and ALP levels in the MCD+infliximab group.

Contrary to the studies of Koca et al. and Barbuio et al., infliximab was not associated with a significant biochemical and histopathological improvement in our study. The study by Koca et al. also did not report a significant improvement in hepatocyte ballooning, in contrast to our observation of a significant improvement in hepatocyte ballooning in the MCD+infliximab group (27,28).

There have been no previous reports using adalimumab in NASH models. However, Schramm and colleagues reported that AST, ALT, and γ-glutamyl transpeptidase levels were decreased to normal levels in a patient with rheumatoid arthritis and concomitants NASH upon treatment with adalimumab (22). They did not provide a histopathological evaluation in their case study.

In our study, TNF-α levels were significantly suppressed in the group that received adalimumab compared to the MCD group. Moreover, the TNF-α levels in the adalimumab group were lower than those in the control group, which was fed anormal diet and did not have NASH. This finding suggests that adalimumab is highly effective in the suppression of TNF-α. In addition, adalimumab demonstrate significantly higher suppression of TNF-α when compared with the other treatments (infliximab and pentoxifylline).

Adalimumab also leads to significant suppression of IL6 and TGF-β. IL8 also suppressed, but not significantly compared to the MCD group.

The steatosis score was 3 in both the MCD and MCD+adalimumab groups. However, hepatocyte ballooning was significantly decreased in the adalimumab group. The mean total NASH score was decreased in the adalimumab group, but this decrease was not significant. There were no significant differences in biochemical parameters (AST, ALT, ALP, bilirubin, albumin, and total protein) between the MCD and MCD+adalimumab groups. However, AST, ALT, and ALP levels demonstrated a slight decrease in the MCD+adalimumab group.

In their study of 9 NASH patients, Satapathy et al. determined that AST and ALT levels were significantly reduced after 12 months of pentoxifylline treatment. In addition, a decline in fibrosis was observed in 5 patients (24).

Lee and colleagues performed a randomized controlled study in which, 20 NASH patients were divided in two (groups: one treated with pentoxifylline (400 mg three times a day) and the other with placebo, along with diet and exercise). After 3months of treatment, the group receiving pentoxifylline demonstrated a significant decrease in AST levels along with non-significant decreases in. ALT, TNF-α, and IL-6 levels as compared to placebo (25). Histopathological evaluation was not performed at the end of the study.

Yalniz et al. studied the effects of pentoxifylline on a NASH model that was created by administering rats a high-fat diet for 4 weeks**.** They determined that after 2 weeks of 4.5 mg/kg/day intraperitoneal pentoxifylline treatment, AST and ALT levels were significantly decreased in the pentoxifylline group compared to the group receiving placebo. When evaluated histopathologically, the density of steatosis inflammatory cells (/mm^3) and ballooning degeneration were significantly lower in pentoxifylline group (26).

In our study, the MCD + pentoxifylline group demonstrated significant suppression of serum TNF-α levels compared to the MCD group. TNF-α levels in the pentoxifylline group were similar to those of the non-NASH normal diet group indicating that pentoxifylline was effective at suppressing TNF-α. Pentoxifylline was also foundto significantly suppress IL6, IL8, and TGF-β levels. There was no difference in steatosis, as both the pentoxifylline group and the MCD group had a steatosis score of 3. Pentoxifylline appeared to suppress lobular inflammation and hepatocyte ballooning, but this suppression was not significant. Finally, the pentoxifylline group demonstrated a non-significant decrease in the total NASH score.

There were no significant differences in biochemical parameters (AST, ALT, ALP, bilirubin, albumin, and total protein) between the MCD and the MCD + pentoxifylline groups. However, there was a slight decrease in AST, ALT, and ALP levels in the MCD + pentoxifylline group. Our study showed that these three anti-TNF-α agents could suppress TNF-α levels in our model. Despite effectively suppressing TNF-α, these agents were not able to prevent the development of NASH. However, they were able to slightly improve NASH histopathology scores and exert a non-significant beneficial effect on liver function. Histopathologically and biochemically, there were no significant differences between the three agents. These data suggest that suppression of TNF-α, which is a cytokine involved in the pathogenesis of NASH, does not prevent the development of NASH, and does not have any significant effect on the treatment of NASH. The pathogenesis of NASH is multi-factorial, and TNF-α suppression has limited merits in the treatment of NASH.

In conclusion, our study showed that all three anti-TNF agents were able to successfully suppress TNF-α, but were unable to prevent the development of NASH. However, they were able to slightly improve NASH histopathological score and had non-significant positive effects on liver function.

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