RESEARCH ARTICLE

Interleukin-10 contribute to the healing by correcting the proinflammatory status in obstructive jaundice endotoxemia

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Abstract

Background: This study aims to elucidate the beneficial effects of Interleukin-10 (IL-10) on ameliorating endotoxemia-induced cellular immune suppression and inflammation in obstructive jaundice (OJ), a condition associated with high mortality.

Material and Methods: This study employed a rat model of OJ, characterized by significant morbidity and mortality due to septic complications and disease pathophysiology. The experimental design involved two primary groups of rats, each subdivided into three subgroups. The first primary group comprised rats undergoing sham surgery, sham surgery with endotoxin and physiological serum solution, and sham surgery with endotoxin and interleukin 10 (IL-10). The second primary group included rats with ligated common bile ducts, ligated common bile ducts with endotoxin and physiological serum solution, and ligated common bile ducts with endotoxin and IL-10. We collected blood samples to assess tumor necrosis factor-alpha (TNF-α), alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total/direct bilirubin levels. Additionally, liver tissues were examined for cellular changes. We performed statistical analyses utilizing Kruskal-Wallis and Mann-Whitney U tests.

Results: A statistically significant elevation in serum TNF-α levels was observed in the subgroups with OJ and endotoxin toxicity. Conversely, a significant reduction in TNF-α levels was noted in subgroups treated with IL-10. Furthermore, IL-10 administration markedly attenuated the adverse hepatic effects of OJ and endotoxemia.

Conclusions: IL-10 effectively modulates the proinflammatory state induced by endotoxemia in OJ, as evidenced by reduced TNF-α levels and improved biochemical and cellular markers. The potential clinical application of IL-10 in OJ could contribute to lowering mortality rates. HIPPOKRATIA 2024, 28 (4):165-172.

Keywords: Interleukin-10, endotoxemia, obstructive jaundice, tumor necrosis factor

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Introduction

Obstructive Jaundice (OJ) is a clinical condition resulting from the obstruction of bile flow due to extrahepatic biliary pathologies, including bile duct stones, traumatic injury (interventional, endoscopic, or surgical), tumors, or pancreatic pathologies such as pancreatic cancer and pancreatitis[1]. This obstruction leads to the weakening of the intestinal barrier and endotoxemia due to bacterial translocation. The systemic inflammatory response triggered by endotoxins can lead to serious complications such as sepsis and multi-organ failure, significantly contributing to morbidity and mortality[2-5]. Also, the presence of cellular immune dysfunctions and the proliferation of toxic substances, such as bilirubin, in OJ patients contribute to this process[3-8].

The association between OJ and endotoxemia is predominantly related to the dysfunction of the liver and subsequent liver detoxification function's impairment. In OJ, bile acids and bilirubin accumulation result in hepatocyte damage, thus impairing the liver's ability to metabolize and purify endotoxins effectively. Elevated serum liver enzyme levels, such as alanine aminotransferase (ALT) and alkaline phosphatase (ALP), are often observed in patients with OJ, denoting hepatocellular damage and cholestasis[9]. This hepatic dysfunction, combined with the increased permeability of the intestinal barrier, permits bacteria and their products to enter the bloodstream, thus contributing to endotoxemia[10].

An additional crucial factor in OJ's pathophysiology is the alteration in Kupffer cell activation in the liver that can result in portal and systemic endotoxemia and hepatocellular damage[6,11,12]. Human and animal model studies have demonstrated that Kupffer cell dysfunction becomes evident in endotoxemia[12]. Also, alterations in Kupffer cells' activity, together with imbalances in immune mediators such as fibronectin and plasma complement

levels, contribute to hepatocellular damage. This study administered endotoxin to an OJ rat model to evaluate the inflammatory response induced by endotoxemia. Endotoxin triggers the release of proinflammatory cytokines from macrophages, notably tumor necrosis factor (TNF), playing a prominent role in the inflammatory cascade[13,14].

Various studies documented that proinflammatory cytokines, particularly TNF-alpha (TNF-α), are significantly elevated in response to OJ-associated endotoxemia[11,14,15]. TNF-α elevation is crucial as it participates in sepsis's underlying inflammatory processes. Hence, investigations in recent years focused on blocking TNF-α as a therapeutic strategy[15-18]. TNF-α elevation released by monocytes/macrophages and Kupffer cells significantly affects the critical response to endotoxin in experimental OJ[19,20]. TNF-α is even considered the primary mediator of the observed metabolic changes following endotoxin injection[5,11,14,15]. TNF-α reaches its peak concentration within two hours after endotoxin administration and its half-life is 18.2 minutes[21].

The injection of TNF-α into experimental animals replicates the clinical symptoms typically observed following endotoxin administration. The clinical symptoms induced by endotoxin and cytokine treatments can be alleviated by anti-endotoxin and anti-cytokine therapies[8,22]. It has been shown that anti-TNF antibodies, such as rosiglitazone, and TNF inhibition therapies enhance the anti-inflammatory response in endotoxemia by reducing TNF release from macrophages in vitro[23].

Interleukin-10 (IL-10), a cytokine produced by various immune cells, including T helper-1 and T helper-2 lymphocytes, monocytes/macrophages, mast cells, and B lymphocytes[19,24], has the primary function of inhibiting cytokine production in both T cells and natural killer (NK) cells, achieved indirectly by suppressing the functions of accessory cells, mainly monocytes and macrophages[25]. IL-10 suppresses inflammation by reducing proinflammatory cytokines synthesis such as TNF-α, IL-1a, IL-1b, IL-18, and IL-8 activated by endotoxin in monocytes/macrophages[25].

As an anti-inflammatory cytokine, IL-10 is critical in mitigating the inflammation caused by endotoxemia by suppressing the release of proinflammatory cytokines such as TNF-α[26,27]. Moreover, IL-10, also known as cytokine synthesis inhibitory factor, has been shown in experimental studies to regulate inflammation in the liver sinusoids in response to endotoxemia released from Kupffer cells[19, 20,22,28]. Furthermore, it is suggested that IL-10 may reduce morbidity and mortality by regulating the development of the compensatory anti-inflammatory response syndrome, a critical phase where most deaths in sepsis occur[6,11,16,17]. Over the past 50 years, clinical studies have shown that sepsis treatments aimed at alleviating symptoms of systemic inflammatory response syndrome have yielded suboptimal results, indicating a need for alternative therapeutic approaches[29].

Research has demonstrated that inhibiting IL-10 activity during the early stages of sepsis does not lead to an increase in mortality; however, blocking IL-10 activity beyond 12 hours significantly raises mortality rates[5,30]. Clinical applications of IL-10 in immunotherapy for inflammatory bowel diseases, allergic diseases, acute pancreatitis, breast cancer, and squamous cell carcinoma have been reported[5,19,25,26,31,32].

Given the high mortality and morbidity associated with OJ, there is an urgent need to develop interventions that can modulate the proinflammatory state induced by endotoxins. If IL-10 can effectively counteract the harmful effects of endotoxemia by regulating cytokine activity, it may offer a novel therapeutic approach for managing endotoxemia associated with OJ. The present study aims to investigate whether IL-10 can improve the proinflammatory state and support biochemical and pathological recoveries in tissues by evaluating its effects on serum TNF-α levels and liver histopathology in an experimental model of endotoxemia associated with OJ, a subject not comprehensively studied.

Material and Methods

Study design

Following approval of the Gazi University Faculty of Medicine Animal Experiments Ethics Committee, we conducted a randomized, double-blind, placebo-controlled experimental study in the Surgical Experimental Research Laboratory. A total of fifty-four male Wistar-Albino rats, weighing between 150 and 220 grams, were utilized in this research. The animals were acclimated to the laboratory environment one week prior to the commencement of the study, maintained on a standard diet, and housed in cages under controlled conditions at 21 ± 2 °C with a 12-hour light-dark cycle. Water and feed were withheld 12 hours before surgical procedures, and no antibiotics were administered. No sample size calculation was performed; since this study is an experimental model, the sample size was determined based on the number of animals used in similar studies in the literature. Therefore, at least six animals were included in each group to ensure that each group had sufficient statistical power[33].

Surgical procedures

We randomly divided rats into two primary groups: the sham-operated group (n =24) and the experimental group with OJ (n =30), and further subdivided each primary group into three subgroups. We performed all surgical procedures under sterile conditions and induced anesthesia with intramuscular injection of 50 mg/kg ketamine hydrochloride (Ketalar R; Parke Davis, USA).

At the sham-operated group, we performed a 1.5-2 cm supraumbilical midline laparotomy and mobilized the supraduodenal common bile duct without ligation. In contrast, in the OJ group, the supraduodenal common bile duct was ligated with 5/0 nonabsorbable sutures, using a double ligature proximally and a single ligature distally. Each primary group was subdivided as follows (Figure 1): Group 1A is the sham surgery group; Group 1B is the sham surgery plus endotoxin plus physiological saline

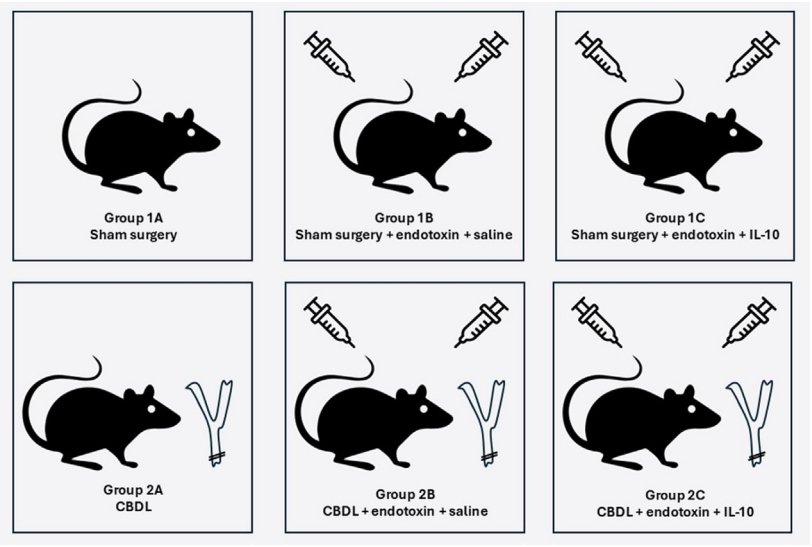

Figure 1: Schematic graph demonstrating the rat subgroups' features.
CBDL: Common bile duct ligation.

group; Group 1C is the sham surgery plus endotoxin plus IL-10 group; Group 2A is the common bile duct ligation group; Group 2B is the common bile duct ligation plus endotoxin plus physiological saline group; and Group 2C is the common bile duct ligation plus endotoxin plus IL-10 group.

Experimental procedures

On the fifth postoperative day, endotoxemia was induced in the respective subgroups by administering *Escherichia coli* serotype 055 endotoxin (Sigma Chemical Co., St. Louis, MO, USA) via the tail vein at a sublethal dose of 200 μg/kg. Thirty minutes after endotoxin administration, rats in subgroups 1C and 2C received 100 ng/ml recombinant rat IL-10 (MyBiosource Int., CA, USA) intraperitoneally, whereas subgroups 1B and 2B received an equivalent volume of physiological saline. The fifth postoperative day was selected as it is defined in the literature as the time when the effects of endotoxemia and OJ are most pronounced[34].

Sample collection

Following the treatment regimen, rats were re-anesthetized, and blood samples were collected via intracardiac puncture 60 minutes post-IL-10 or saline administration and 90 minutes post-endotoxin administration. We also collected liver tissue samples from the median lobe for histopathological examination. We centrifuged blood samples at 3,000 rpm for five minutes and stored the serum at -70 °C for subsequent biochemical and TNF-α assays.

Biochemical and cytokine assays

Serum TNF-α levels were quantified using a rat TNF-α ELISA kit (Biosource International, CA, USA). We measured biochemical parameters using a commercial biochemical kit (Menarini, Italy), including aspartate aminotransferase (AST), ALT, ALP, total bilirubin, and direct bilirubin levels. We determined total and direct bilirubin levels using the Jendrassik-Graft method.

Histopathological examination

We fixed liver tissue samples in 10 % formalin and processed them for routine histological examination. We embedded samples in paraffin, sectioned, and stained with hematoxylin-Eosin, Periodic Acid-Schiff (PAS), D-PAS, bilirubin, and iron pigment dyes. A single pathologist blindly performed the histopathological evaluations, grading the findings under a light microscope.

Statistical analysis

We calculated the mean ± standard error of the mean (SEM) of biochemical values for each subgroup using the IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA). We employed the Kruskal-Wallis one-way analysis of variance to assess differences between the sham and study groups. Using the Mann-Whitney U test, we conducted pairwise comparisons between subgroups (e.g., 2A-2B, 1B-2B). We defined statistical significance as $p < 0.05$. Non-parametric tests were selected because the data did not meet the normality assumptions. We tested compliance with normal distribution with the Shapiro-Wilk test, and since the data did not show normal distribution, Kruskal-Wallis and Mann-Whitney U tests were used[35].

Results

Observations

This study observed clinical signs of jaundice in groups 2A, 2B, and 2C starting the second day after the common bile duct ligation. By the fifth day, these rats exhibited yellow-green discoloration in the peritoneum and proximal common bile duct dilation.

TNF-α levels and other biochemical results

Table 1 presents the mean (±SEM) serum TNF-α levels of the subgroups. Analysis using the Kruskal-Wallis one-way analysis of variance demonstrated significant differences in TNF-α levels within each primary group (p =0.0001 for both groups). Pairwise comparisons using the Mann-Whitney U test revealed significant differences in TNF-α levels between subgroups 1A-1B, 1B-1C, and 1A-1C (p =0.0006, p =0.0157, and p =0.0006, respectively). Similarly, significant differences were observed between subgroups 2A-2B, 2B-2C, and 2A-2C (p =0.0001, p =0.0025, and p =0.0001, respectively). On the other hand, we did not detect a significant difference in the TNF-α levels between subgroups 1A-2A, 2B-2C, and 3A-3B.

The significant increase in AST levels observed in subgroup 1A is thought to be related to the mild inflammatory response that developed after sham surgery.

The mean (±SEM) biochemical values of the subgroups are detailed in Table 1. Statistical analysis using the Kruskal-Wallis one-way analysis of variance and the Mann-Whitney U test demonstrated significant differences in several biochemical parameters, as shown in Table 2, Figure 2.

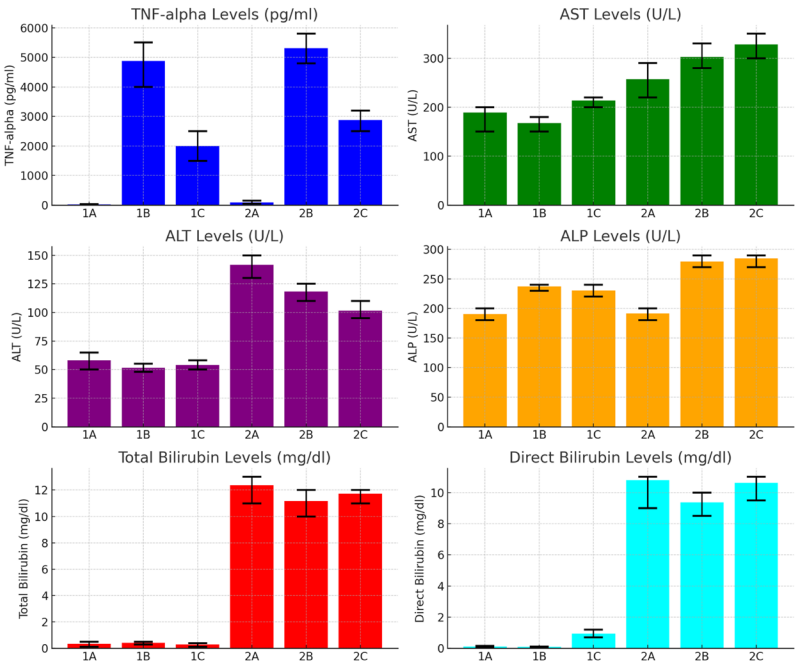

Figure 2: Composite image with graphs demonstrating TNF-α and other biochemical parameters regarding each of the subgroups.

Subgroup 1A: sham surgery, Subgroup 1B: sham surgery plus endotoxin plus physiological saline, Subgroup 1C: sham surgery plus endotoxin plus IL-10, Subgroup 2A: common bile duct ligation, Subgroup 2B: common bile duct ligation plus endotoxin plus physiological saline, Subgroup 2C: common bile duct ligation plus endotoxin plus IL-10. TNF-α: tumor necrosis factor-α (pg/ml), AST: aspartate aminotransferase (U/L), ALT: alanine aminotransferase (U/L), ALP: alkaline phosphatase (U/L), T.Bil: total bilirubin (mg/dl), D.Bil: direct bilirubin (mg/dl).

Table 1: : Tumor necrosis factor-α levels and other biochemical results of the three sham-operated and three experimental subgroups included in this randomized experimental study

Sub-group	TNF-α (pg/ml)	AST	ALT	ALP	Total bilirubin	Direct bilirubin
1A	21 ± 5.6	188.88 ± 10.82	58 ± 8.61	190.75 ± 10.81	0.33 ± 0.36	0.12 ± 0.01
1B	4876.5 ± 789.3	167.5 ± 13.3	51.62 ± 2.54	237.13 ± 12.67	0.41 ± 0.03	0.1 ± 0.02
1C	2000.8 ± 445.9	213.76 ± 12.18	54 ± 1.89	230.38 ± 14.83	0.28 ± 0.31	0.93 ± 1.17
2A	88.1 ± 26.6	256.7 ± 35.36	141.7 ± 26.2	191.4 ± 12.91	12.37 ± 1.3	10.79 ± 1.17
2B	5314 ± 557.9	302.2 ± 28.69	118.4 ± 11.49	279.7 ± 24.77	11.16 ± 0.7	9.37 ± 0.7
2C	2880 ± 226.6	327.7 ± 32.01	101.6 ± 8.03	284.6 ± 38.11	11.74 ± 0.47	10.62 ± 0.5

Values are presented as mean ±standard error of the mean. Subgroup 1A: sham surgery, Subgroup 1B: sham surgery plus endotoxin plus physiological saline, Subgroup 1C: sham surgery plus endotoxin plus IL-10, Subgroup 2A: common bile duct ligation, Subgroup 2B: common bile duct ligation plus endotoxin plus physiological saline, Subgroup 2C: common bile duct ligation plus endotoxin plus IL-10. TNF-α: tumor necrosis factor-α (pg/ml), AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase.

Histopathological findings

Liver parenchymal changes were examined blindly by a single pathologist in terms of the 12 features listed in Table 3, who evaluated semiquantitatively the histological findings. Table 3 shows the median values of liver histological findings in grades according to subgroups.

In subgroup 2A rats, moderate parenchymal and inflammatory changes were observed, while severe bile duct changes and mild fibroblastic activity were detected. Apart from moderate fibroblastic activity in subgroup 2B rats, parenchymal, inflammatory, and bile duct changes were severe (Figure 3). In subgroup 2C rats administered endotoxin plus IL-10, severe bile duct changes, moderate necrotic changes, mild parenchymal changes, and inflammatory changes were found (Figure 4). Fibroblastic activity was not detected in this subgroup.

Parenchymal changes, inflammatory changes, bile duct changes, and fibroblastic activity were close to normal in subgroup 1A rats. In subgroup 1B rats, parenchymal changes were moderate, inflammatory and bile duct changes were mild, and no fibroblastic activity was observed. While parenchymal and inflammatory changes were partially mild in subgroup 1C rats, no bile duct changes or fibroblastic activity were observed.

When we statistically evaluated the liver histological findings using Kruskal-Wallis one-way analysis of variance, we detected a significant difference in all evaluations except for fibroblastic activity in the sham group and bile duct changes in the study group.

A significant difference was observed in all hepatic findings, except bile duct changes, between subgroups 2B-2C (Mann-Whitney-U test). It was determined that histological findings improved in many parameters in subgroup 1C, given endotoxin plus IL-10, compared to subgroup 1B, given only endotoxin.

The results of comparing the subgroups in pairs with the Mann-Whitney U test in terms of significant differences are given in Table 4.

Table 2: Level of significance (p value) for the comparison of the biochemical values within the primary groups and between the subgroups.

	Main groups		**Subgroups**								
	1st Group (1A-1B-1C)	**2nd Group (2A-2B-2C)**	**1A-1B**	**1B-1C**	**1A-1C**	**2A-2B**	**2B-2C**	**2A-2C**	**1A-2A**	**1B-2B**	**1C-2C**
TNF-α	**0.0001**	**0.0001**	**0.0006**	**0.0157**	**0.0006**	**0.0001**	**0.0025**	**0.0001**	0.1295	0.8590	0.1309
AST	**0.0454**	0.4396	0.2076	**0.0274**	0.0929	0.4497	0.6501	0.1988	0.2481	**0.0014**	**0.0077**
ALT	0.4354	0.3036	0.8738	0.3991	0.1873	0.7913	0.3254	0.1124	**0.0016**	**0.0004**	**0.0004**
ALP	0.0651	**0.0058**	**0.0357**	0.8334	0.0587	**0.0052**	0.9698	**0.0065**	0.8590	0.2665	0.2863
T. Bil	0.0761	0.6781	0.2929	**0.0272**	0.2069	0.6501	0.4963	0.4497	**0.0004**	**0.0004**	**0.0004**
D. Bil	0.4788	0.4395	0.3692	0.9157	0.2434	0.4961	0.2899	0.3256	**0.0004**	**0.0004**	**0.0004**

Kruskal-Wallis one-way analysis of variance assessed differences between the main groups while pairwise comparisons between subgroups were assessed with the Mann-Whitney U test. p values less than 0.05 are in bold text. Subgroup 1A: sham surgery, Subgroup 1B: sham surgery plus endotoxin plus physiological saline, Subgroup 1C: sham surgery plus endotoxin plus IL-10, Subgroup 2A: common bile duct ligation, Subgroup 2B: common bile duct ligation plus endotoxin plus physiological saline, Subgroup 2C: common bile duct ligation plus endotoxin plus IL-10. TNF-α: tumor necrosis factor-α (pg/ml), AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, T.Bil: total bilirubin, D.Bil: direct bilirubin.

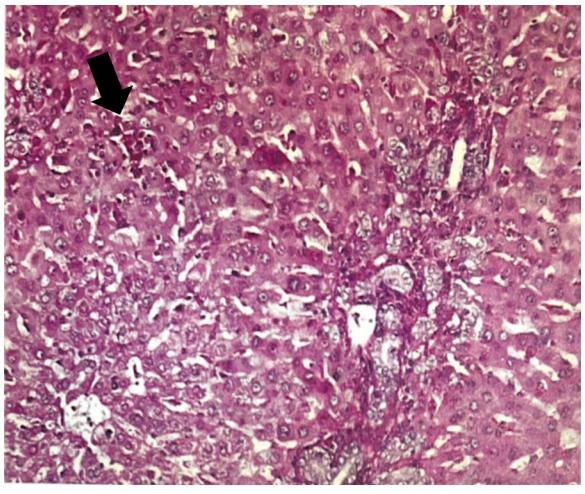

Figure 3: Histopathology image of subgroup 2B liver findings with severe parenchymal and portal findings (Periodic acid–Schiff stain, x 20). Apart from moderate fibroblastic activity, severe parenchymal, inflammatory, and bile duct changes are observed. An arrow indicates an area of focal necrosis. The findings are reported in Table 3.

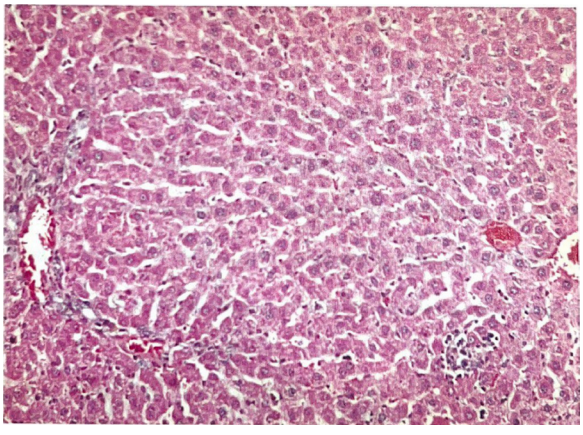

Figure 4: Histopathology image of subgroup 2C liver findings with mild parenchymal and portal findings (Hematoxylin-Eosin stain, x 20). Except for moderate necrotic changes, mild parenchymal, inflammatory, and severe bile duct changes are shown. Fibroblastic activity was not detected. The findings are reported in Table 3.

Table 3: Median values of liver histological findings evaluated blindly and semiquantitatively by a single pathologist in terms of the 12 listed features according to subgroups.

	1A	1B	1C	2A	2B	2C
Kupffer cell hyperplasia	0	2	1	2	3	1
Hydropic degeneration	0	2	1	2	3	1.5
Steatosis	0	1.5	0	2	2.5	0
Necrotic changes	0	2	1.5	1.5	3	2
Hepatocyte Atrophy	0	1	0	2	3	1
Sinusoidal congestion	0	1	1	2	3	1
Fibrin thrombus	0	1	0	0	1	0
Sinusoidal inflammation	0	1	1	2	3	1
Endothelialitis	0	1	1	1	3	1
Portal inflammation	0	1	1	2	3	1
Bile duct changes	0	1	0	3	3	3
Fibroblastic activity	0	0	0	1	2	0

Subgroup 1A: sham surgery, Subgroup 1B: sham surgery plus endotoxin plus physiological saline, Subgroup 1C: sham surgery plus endotoxin plus IL-10, Subgroup 2A: common bile duct ligation, Subgroup 2B: common bile duct ligation plus endotoxin plus physiological saline, Subgroup 2C: common bile duct ligation plus endotoxin plus IL-10.

Table 4: Level of significance (p value) for the comparison of the results of liver histological examinations within the primary groups and between the subgroups.

	Main groups		Subgroups					
	1st Group (1A-1B-1C)	2nd Group (2A-2B-2C)	1A-1B	1B-1C	1A-1C	2A-2B	2B-2C	2A-2C
Kupffer cell hyperplasia	**0.0001**	**0.0001**	**0.0002**	**0.0027**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Hydropic degeneration	**0.0001**	**0.0001**	**0.0002**	**0.0455**	**0.0002**	**0.0001**	**0.0001**	**0.0118**
Steatosis	**0.0001**	**0.0001**	**0.0003**	**0.0017**	0.1432	**0.0118**	**0.0001**	**0.0001**
Necrotic changes	**0.0001**	**0.0001**	**0.0001**	**0.0253**	**0.0003**	**0.0001**	**0.0001**	**0.0118**
Hepatocyte Atrophy	**0.0002**	**0.0001**	**0.0001**	**0.0027**	0.1432	**0.0004**	**0.0001**	**0.0011**
Sinusoidal congestion	**0.0001**	**0.0001**	**0.0001**	**0.9999**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Fibrin thrombus	**0.0001**	**0.0001**	**0.0001**	**0.0001**	0.9999	**0.0001**	**0.0001**	0.9999
Sinusoidal inflammation	**0.0002**	**0.0001**	**0.0002**	0.0531	**0.0027**	**0.0007**	**0.0001**	**0.0038**
Endothelialitis	**0.0002**	**0.0001**	**0.0002**	0.0528	**0.0027**	**0.0001**	**0.0002**	0.1951
Portal inflammation	**0.0001**	**0.0001**	**0.0002**	0.1709	**0.0006**	**0.0001**	**0.0001**	**0.0073**
Bile duct changes	**0.0001**	0.9999	**0.0001**	**0.0001**	0.9999	0.9999	0.9999	0.9999
Fibroblastic activity	0.3679	**0.0001**	0.3173	0.3173	0.9999	**0.0001**	**0.0001**	**0.0001**

Kruskal-Wallis one-way analysis of variance assessed differences between the main groups while pairwise comparisons between subgroups were assessed with the Mann-Whitney U test. p values less than 0.05 are in bold text. Subgroup 1A: sham surgery, Subgroup 1B: sham surgery plus endotoxin plus physiological saline, Subgroup 1C: sham surgery plus endotoxin plus IL-10, Subgroup 2A: common bile duct ligation, Subgroup 2B: common bile duct ligation plus endotoxin plus physiological saline, Subgroup 2C: common bile duct ligation plus endotoxin plus IL-10.

Discussion

This study investigates the therapeutic potential of IL-10 in addressing the proinflammatory state induced by endotoxemia using an experimental OJ model. According to our findings, IL-10 administration significantly decreased serum TNF-α levels and substantially enhanced biochemical and histopathological parameters, suggesting that IL-10 could effectively treat OJ-associated endotoxemia.

OJ is a severe clinical condition that results from bile duct obstruction, leading to impaired liver function and raised systemic endotoxin levels. The reticuloendothelial system damage results in decreased liver detoxification capacity and, thus, endotoxin clearance reduction, which, combined with the increased intestinal permeability, results in high endotoxin levels in these patients[9]. Notably, the increase in endotoxin levels is more pronounced in clinical conditions like prolonged biliary obstructions and malignancies[10,36]. Researchers, prompted by OJ's severe clinical picture, investigate treatments for this condition.

IL-10 is a potent anti-inflammatory cytokine that inhibits proinflammatory cytokine release and suppresses the inflammatory response[37,38]. Also, it can potentially reduce liver damage by suppressing inflammatory reactions triggered by endotoxins[38]. Therefore, the external administration of IL-10 may be beneficial in controlling the inflammatory responses associated with endotoxemia in obstructive jaundice. Secreted by Kupffer cells, IL-10 plays a critical role in maintaining liver homeostasis and regulating immune responses[39]. Studies have shown that IL-10 reduces inflammation by inhibiting the production

of proinflammatory cytokines such as TNF-α and IL-6, and by suppressing hepatocyte apoptosis, it supports liver tissue repair[40,41]. Additionally, IL-10 is known to promote tissue healing by suppressing hepatocyte apoptosis. These effects of IL-10 have brought up its potential use in treating the clinical picture caused by endotoxemia.

In this study, endotoxemia was induced by administering 200 mcg of lipopolysaccharide (LPS) through the tail artery, simulating intestinal endotoxemia, and mimicking bile duct manipulation in the presence of extrahepatic OJ. As a result, consistent with the existing literature, we observed statistically significant increases in serum TNF-α levels in both the sham group and the obstructive jaundice group after LPS administration. This result confirms that LPS is one of the most effective agents in inducing TNF-α release, as previously reported in the literature[18]. Furthermore, similar to previous studies, elevated levels of TNF-α and IL-10 were reported in rats subjected to experimental endotoxemia, regardless of OJ presence[28]. Although the difference was not statistically significant in our study, the fact that the OJ group had higher TNF-α levels than the sham group is important in showing that obstructive jaundice brings an endotoxin load.

Another important contribution of this study is demonstrating that the therapeutic application of IL-10 significantly reduces TNF-α levels. IL-10 administration significantly reduced TNF-α in both the sham and OJ groups, demonstrating IL-10's therapeutic potential in clinical conditions with elevated TNF-α levels.

Subgroup results comparisons indicate LPS as a potent agent in inducing TNF-α release while IL-10 administration leads to significant TNF-α level reduction. Moreover, the clinical potential of IL-10 is highlighted by the cytokine's positive effects on hepatocyte protection, inflammatory response modulation, and liver regeneration, suggesting IL-10 offers a promising therapeutic approach to managing inflammation and promoting liver recovery in elevated TNF-α clinical conditions.

Pathology examinations demonstrated the adverse effects of both endotoxins and OJ on the liver, with the most severe findings observed in liver tissue samples of the group subjected to the OJ model and endotoxin administration. Importantly, we found that IL-10 treatment reduced liver damage significantly, as histopathological findings significantly improved in the OJ group receiving IL-10 compared to the control group. Previous studies demonstrated IL-10's hepatoprotective effect in different clinical conditions[42]. Our study clearly shows the hepatoprotective therapeutic potential of IL-10 in reducing liver inflammation and supporting tissue repair in OJ.

Despite the promising results, our study has several limitations. Future research on IL-10 knockout rats could provide more detailed insights regarding the mechanisms of exogenous IL-10 in OJ-induced sepsis. Additionally, measurements of serum IL-2, another cytokine known to be elevated in OJ, would offer a broader understanding of the immune response[28]. Including groups receiving corticosteroids and IL-10, as well as varying doses and administration timings, would provide valuable information[5]. Finally, the appropriate timing and dosage of IL-10 administration in rats remain debatable. Additional research is required to define the optimal conditions for anti-cytokine treatment in endotoxemia, particularly regarding patient selection and timing[5,30].

During endotoxemia, IL-10 serves as a critical anti-inflammatory cytokine whose administration in experimental models leads to proinflammatory cytokine level decrease, increased survival rates, and a potential shift toward a more reparative phenotype in macrophage activation. IL-10 modulation in the clinical setting may provide a promising therapeutic strategy for managing endotoxemia- and sepsis-associated inflammatory responses. Specifically, exogenous IL-10 treatment in the OJ context shows the potential to control inflammatory responses, reduce liver damage, and enhance recovery.

Conclusion

We investigated the IL-10 effects on serum levels of the proinflammatory cytokine TNF-α and tissues following experimental endotoxemia in an OJ model, which is associated with high mortality and morbidity. Our findings indicate that IL-10 i) significantly reduces TNF-α levels, a key mediator of endotoxemia toxicity, in the presence and absence of OJ, and ii) improves liver function and morphology regardless of OJ presence in an experimental endotoxemia model.

Based on these results, we concluded that IL-10 ameliorates the adverse effects of both jaundice and endotoxemia in OJ. Future research should focus on determining the optimal dose and timing of IL-10 administration for both therapeutic and prophylactic purposes in OJ. IL-10 is anticipated to become an integral part of clinical practice for managing OJ.

Conflict of interest

Authors declare no conflicts of interest.

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