used in treatment of inflammatory bowel diseases for approximately 50 years [4, 5]. 5-ASA is a small hydrophilic organic acid and is well absorbed from the intestines. It is rapidly transformed to N-acetyl in the intestinal epitelium and liver, and the inactive molecule N-acetyl-5-ASA is generated. Some parts of the inactive metabolite are secreted back to the lumen and removed from the body by feces. Some parts of 5-ASA are metabolized in the liver and extracted by the kidney. However, the clinical effect of 5-ASA is not associated with systemic absorption and redistribution on target organs; its topical effect is observed in the colon. There are various views on the effect mechanisms of 5-ASA compelexes. It was stated that 5-ASA complexes inhibit IL-1 and IL-2 synthesis in the inflammatory period. It was also proved that they inhibit TNF-α synthesis [6].

The aim of the present study was to investigate the effect of intra-abdominally administered mesalazine on cytokines (TNF-α, IL-1β, and IL-6) and on the level of acute-phase reactant CRP during sepsis due to secondary peritonitis.

MATERIALS AND METHODS

Animal study

The study was conducted at the Institute of Experimental Medicine, Istanbul Faculty of Medicine, Istanbul University. The study protocol was approved by the Animal Care Ethics Committee. All procedures were conducted in accordance with the ethics guidelines for the treatment and welfare of experimental animals by the Istanbul Faculty of Medicine, Istanbul University, and Helsinki decleration. In total, 24 male Sprague–Dawley rats weighing 250–280 g were used in this study. The animals were housed at 21°C and were given tap water and standard rat food ad libitum.

Surgical procedure

The animals were anesthetized via an intramuscular injection of ketamine hydrochloride (50–100 mg/kg of body weight). Twenty-four male rats were randomly assigned to three groups. Group I, no irrigation after the development of peritonitis; Group II, irrigation using isotonic solution in the 24th hour after the development of peritonitis; and Group III, irrigation using mesalazine solution in the 24th hour after the development of peritonitis.

Peritonitis was generated by small holes on the cecums of rats using a 22-G needle after laparotomy. Ten

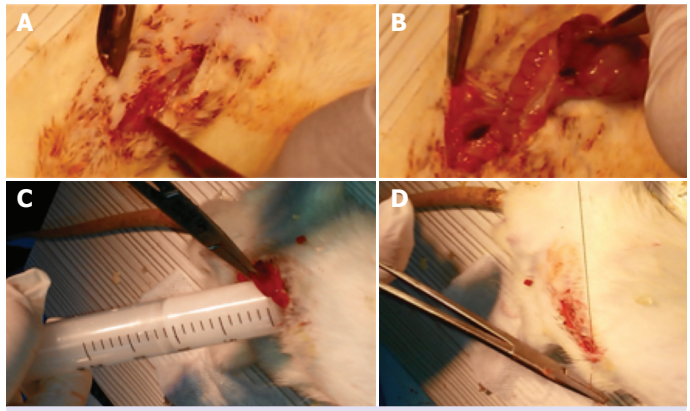

Figure 1. (A, B) Midlinere laparotomy (C) Peritoneal lavage with mesalazine (D) Closure of the abdominal cavity.

milliliter isotonic solution was abdominally administered to Group II and mesalazine (0.2 g/10mL) to Group III 24 h later (Fig. 1). A second laparotomy was performed in the 48th hour. Intracardiac blood samples were taken. Blood samples were collected for the measurement of TNFα, IL-1β, ıL6, and CRP levels. All rats were sacrificed 48 h after the first laparotomy. Tissue samples were taken from the lung.

Cytokines measurement

Samples were collected in serum separator tubes. After clot formation, samples were centrifuged at 1000×g for 10 min, and serum was collected. Serum was stored at −40°C until required for analysis. Cell signal proteins were assessed in the top supernatant of blood by TNF-α, IL-1β, IL-6, and CRP levels using an enzyme-linked immunosorbent assay kit in accordance with the manufacturer's guidelines.

Histologic analysis

Lung tissue samples were taken from the rats for histopathologic investigation because the risk of development of multiple organ failure was mainly in the lung in sepsis. For histologic assessments, lung samples were obtained from eight anesthetized rats is each group at 48 h through excisional biopsy. All specimens were fixed in 10% buffered formalin. Paraffin blocks were prepared from routinely processed specimens, and 5-μm sections were cut and deparaffinized. The sections were stained using hematoxylin–eosin (H&E). Histopathologic examinations were performed by a blinded pathologist with light microscopy at ×200 magnification.

Table 1. Characteristics of groups

	N	Mean	Median	St. Dev.	Min.	Max.
TNF	18	227.4	227.3	27.8	185.1	175.4
IL-6	18	9.8	9.8	1.3	7.9	12.6
IL-1beta	18	1827.8	1767.5	196.9	1556.3	2271.0
CRP	18	184.2	198.1	31.3	128	237.4
Lenfosit	14	48.4	39.5	24.1	9.0	92.6
Nötrofil	13	19.4	11.4	21.7	1.5	63.5
Lökosit	14	6.9	7.2	2.6	3.0	12.3

Table 2. Post-hoc test

		p
TNF	1-2	0.073*
	1-3	0.003*
	2-3	0.038*
IL-1beta	1-2	0.214*
	1-3	0.419*
	2-3	0.914*
CRP	1-2	0.808*
	1-3	0.001*
	2-3	0.114*

*Mann-Whitney U p value.

Statistical method

Descriptive statistics were used to describe continuous variables (mean, standard deviation, minimum, maximum, and median). Comparison of three independent and non-normally distributed continuous variables was performed using the Kruskal–Wallis test. For significant results of the Kruskal–Wallis and Mann–Whitney U test, Bonferroni correction was used as a post-hoc analysis test.

Statistical significance level was set at 0.05. Statistical analyses were performed using MedCalc Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013).

RESULTS

Intracardiac blood samples were taken 48 h after the operation for TNF-α, IL-1β, ıL-6, and CRP measurement. TNF-α, IL-1β, and CRP levels were detected at a significantly lower level in Group III than in the other two groups (Kruskal–Wallis, $p<0.05$) (Table 1). According to the the post-hoc test results, the differences stemmed from Groups I and III (Mann–Whitney U, $p<0.016$, Bonferroni correction) (Table 2).

There is significant difference between TNF and Group. (Kruskal-Wallis $p<0.05$) According to the Post-Hoc test results, the differences stems from 1. and 3. groups. There is significant difference between II-1beta and Group. (Kruskal-Wallis $p=0.041$) According to the Post-Hoc test results, the differences are not caused by two groups. There is significant difference between CRP and Group. (Kruskal-Wallis $p=0.011$) According to the Post-Hoc test results, the differences stems from 1. and 3. groups (Mann-Whitney U $p<0.016$ Bonferroni correction) (Table 3).

The histologic sections were examined under light microscopy and assessed for inflammation in lung tissue, which can be an indirect sign of sepsis. Slight differences of inflammatory reactions were determined between the groups. Group I showed more inflammatory infiltration than Groups II and III. Group III showed relatively lesser inflammatory infiltration than Group II (Fig. 2).

DISCUSSION

Secondary peritonitis is one of the main causes of sepsis and multiple organ failure. Its mortality rate is still high [7]. Elevated plasmacytokine levels have been related to the development of systemic inflammatory response syndrome, sepsis, and organ dysfunction.

TNF-α has been associated with patients at high-risk for sepsis after abdominal surgery and is a predictor of mortality [8, 9]. Qui P et al. [10] investigated the therapeutic role of anti-TNFagents in a meta-analysis study. They searched 15 studies and concluded that anti-TNF agents produced a modest but significant decrease in

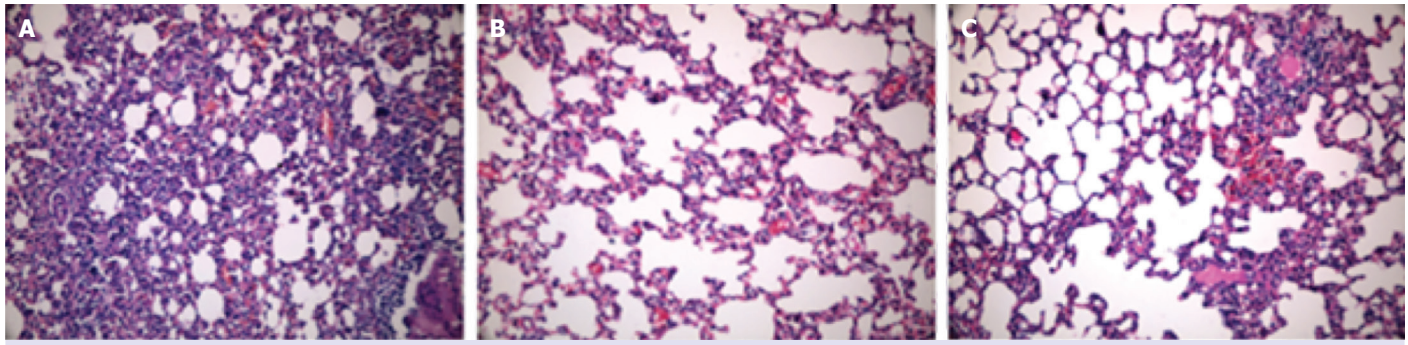

Figure 2. Lung tissue of Group I (A), Group II (B), and Group III (C). Leucocyte infiltration can be seen in Group I. A decrease in leucocyte infiltration can be seen in Groups II and III.

Table 3. Comparison of TNF, IL-1beta, IL-6 and CRP levels parameters according to the groups

	Group	Mean	Median	St. Dev.	Min.	Max.	p	p1	p2	p3
TNF	I	248.8	251.1	21.4	213.9	275.4	<0.05*			
	II	225.1	226.8	13.0	209.8	237.0		0.073	0.003	0.038
	III	200.4	196.3	16.9	185.1	229.9				
IL-1beta	I	1936.2	1933	216.8	1631.2	2271.0	0.041*			
	II	1866.9	1859.2	116.7	1758.8	1990.7		0.214	0.419	0.914
	III	1657.3	1677.3	55.2	1556.3	1710.9				
IL-6	I	9.3	9.4	1.0	7.9	10.6	0.424*			
	II	10.6	10.3	1.7	9	12.6		-	-	-
	III	9.9	9.9	1.3	8.0	12.0				
CRP	I	200.7	202.7	11.1	175.6	210.9	0.011*			
	II	196.3	206.5	43.9	134.9	327.4		0.808	0.001	0.114
	III	154.1	163.4	19.3	128.0	170.5				

*Kruskal Wallis p value; 1I vs. II, 2I vs. III, 3I vs. II (Mann-Whitney U p value).

mortality in patients with sepsis.

High levels of CRP are caused by infections and many inflammatory diseases. The relationship between intra-abdominal infections and CRP is well established [11]. K. Mulari analyzed 66 patients with secondary peritonitis due to gastrointestinal tract perforation and investigated risk factors for hospital mortality. It was concluded that elevated CRP levels and high Mannheim peritonitis index score in the early postoperative phase had a prognostic significance [12].

Peritoneal lavage is described as the washing of the peritoneal space with high volumes of saline. Different agents have been used for peritoneal lavage in the literature. Povidione–iodine (PVI) and saline are well-known agents that are used for this purpose. Araujo ID et al. investigated peritoneal lavage with PVI in rats with experimental peritonitis. Lavage of the peritoneal cavity with PVI demonstrated no beneficial effect in local control of peritonitis [13]. Camargo M et al, showed a beneficial effect of peritoneal lavage with bupovacaine in rats with fecal peritonitis [14]. Peritoneal irrigation was performed in cases of severe pancreatitis and morbidity and mortality rates decreased in this patient group [15]. Coumarin, which has an immunostimulant effect, was used in peritonitis without any beneficial effect [16, 17]. Peritoneal irrigation performed with a local anesthetic agent, 0.2% ropivacarine, in rats with fecal peritonitis decreased the histologic changes that occurred due to inflammation [18].

In our study, Groups II and III underwent the proposed therapeutic process (peritoneal lavage with isotonic solution and mesalazine). The effects of peritoneal lavage with isotonic solution and mesalazine was exam-

ined objectively. The mean CRP level was found statistically significantly lower in Group III than in the other groups. TNFα and IL-1β levels were also lower in Group III. The decrease in TNF-α levels in Group III was statistically significant. We examined the lung tissues to show the systemic effect of intra-abdominal infection. The degree of inflammatory reaction was found lower in Group III, indicating that lavage using mesalazine may have a protective effect on the lung tissue.

CONCLUSION

In rats with intra-abdominal sepsis, TNF-α and CRP levels significantly decreased in the mesalazine group. There were also decrease in leukocyte infiltration in the lungs of rats treated with mesalazine. So, it may be suggested that the use of mesalazine in clinical practice may decrease the sepsis-related morbidity and mortality.

Conflict of Interest: The authors declare no conflict of interest.

Financial Disclosure: The authors declared that this study has received no financial support.

Authorship Contributions: Concept – A.S.; Design – A.S.; Supervision – B.K.; Materials – A.S., M.S.T., L.T.S., B.K.; Data collection &/or processing – L.T.S.; Analysis and/or interpretation – M.S.T., L.T.S., A.S.; Writing – A.S., M.S.T., L.T.S., A.S., B.K.; Critical review – A.S.

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