EXPERIMENTAL STUDY

Effect of intraperitoneal hypochlorous acid (HOCl) on bacterial translocation in an experimental peritonitis model in rats

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ABSTRACT

BACKGROUND: This study compared the effects of saline, routinely used for intra-abdominal irrigation, with hypochlorous acid (HOCl), which we believe could be suitable for clinical practice in the future, on bacterial translocation in a rat model of peritonitis.

METHODS: Four groups were formed: Sham, Control, cecal ligation and puncture with saline (CLP+SF), and cecal ligation and puncture with hypochlorous acid (CLP+HA), with 11 rats in each group, for a total of 44 rats. One rat in the Control group died and was excluded from the study. The comparison focused on saline, which is routinely used for intra-abdominal irrigation, and HOCl, which is considered a potential option for future clinical use.

RESULTS: A statistically significant difference was observed between the CLP+SF and CLP+HA groups in liver, spleen, and mesenteric lymph node tissue cultures ($p<0.001$, $p=0.004$, and $p=0.001$, respectively). However, no significant difference was found between the CLP+SF and CLP+HA groups in blood cultures ($p=0.181$). Although bacterial growth in blood cultures was numerically lower in the CLP+HA group, the absence of statistical significance between the CLP+HA group and other groups was attributed to the limited sample size and the short duration of the experimental peritonitis/sepsis model. Additionally, enzyme-linked immunosorbent assay (ELISA) results from blood samples showed that the mean levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), both inflammatory markers, did not differ significantly among the groups. This indicates that HOCl significantly reduced bacterial translocation without suppressing the inflammatory response.

CONCLUSION: It is predicted that the widespread use of HOCl in clinical practice could reduce mortality and morbidity in cases of perforation-induced peritonitis of intra-abdominal sepsis, shorten hospital stays, lower the cost of medical treatment, and contribute to the national economy in the healthcare sector.

Keywords: Bacterial translocation; hypochlorous acid; interleukin-6 (IL-6); peritonitis; tumor necrosis factor-alpha (TNF-α).

INTRODUCTION

Intra-abdominal sepsis represents a significant source of morbidity and mortality in hospitals worldwide.[1] In secondary peritonitis, the main principles of treatment are control of the source of infection, reduction of contamination, and prevention of recurrent infections. In advanced peritonitis, all purulent and necrotic residues in the abdominal cavity should be removed by surgical intervention.[2] Although many methods have been attempted to prevent peritonitis and intra-abdominal sepsis, the desired outcomes have not yet been achieved.[3]

The main proinflammatory cytokines in sepsis are tumor ne-

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crosis factor-alpha (TNF-α), interleukin-1 (IL-1), IL-6, and IL-8. IL-6 and IL-8 are released following the secretion of TNF-α and IL-1.[4] The release of TNF-α initiates the migration of neutrophils and monocytes to the site of infection. It also stimulates the release of corticotropin-releasing hormone (CRH) from the hypothalamus, suppresses appetite, and induces fever. TNF-α influences the production of acute-phase reactants in the liver, contributes to phagocytosis in macrophages, and accelerates the release of IL-1. Serum TNF-α levels have been found to increase in parallel with the progression of intestinal ischemia.[5] Treatment strategies aimed at suppressing TNF-α are currently being developed in clinical practice.

IL-6 and TNF-α levels have been found to increase significantly following postoperative complications. Elevated IL-6 levels are also considered indicators of postoperative mortality and morbidity.[6,7]

Hypochlorous acid (HOCl) is a broad-spectrum antibacterial, antiviral, antiseptic, and disinfectant. Studies have shown that HOCl is effective against microorganisms rapidly and at low doses, reduces bacterial contamination, eradicates the biofilm layer formed by microorganisms, kills microorganisms beneath the biofilm, and accelerates wound healing by promoting the proliferation of fibroblasts and keratinocytes involved in the healing process.[8-10]

This study was planned based on the hypothesis that hypochlorous acid can be used to prevent bacterial translocation and subsequent tissue damage in sepsis. In our study, we aimed to investigate the effects of hypochlorous acid in an experimentally induced peritonitis model in rats using both culture and inflammatory parameters.

MATERIALS AND METHODS

Grouping of Experimental Animals

In this study, 44 male Wistar-Albino rats, weighing an average of 350-400 grams and aged 20-24 weeks, were used. The number of subjects was determined by power analysis prior to the study. The rats were maintained under standard laboratory conditions (20-24°C room temperature, 50-60% humidity) and were provided with standard feed and water throughout the study. All rats were fasted for 12 hours before surgical intervention.

The experimental animals were randomly assigned to four groups. The groups and surgical procedures were defined as follows:

1. Sham group (n=11): Rats underwent only abdominal incision.

2. Control group (n=11): Rats underwent cecal ligation and puncture (CLP).

3. CLP + serum physiologic (SP) group (n=11): Group that underwent cecal excision after the cecal ligation and puncture procedure, followed by washing with saline.

4. CLP + hypochlorous acid (HA) group (n=11): Group that underwent cecal excision after the cecal ligation and puncture procedure, followed by washing with saline and hypochlorous acid.

In Group 2, micro- and then macro-perforation of the cecum was intended. In Groups 3 and 4, the cecum was excised to prevent further perforation and contamination, and to ensure that the two groups were comparable.

In all groups, the animals were sacrificed 24 hours later by performing a relaparotomy. Blood and tissue samples, including liver, spleen, and mesenteric lymph nodes, were collected after the procedure.

Materials and Devices Used

- Refrigerated centrifuge: Nüve NF 1200 R, Türkiye
- Ultra-low temperature freezer: Snijders Scientific, Netherlands
- Refrigerator: Uğur USS 748, Türkiye
- Drying oven: Memmert model 600, Germany
- CO_2 incubator: Memmert model INCO2, Germany
- Biological safety cabinet: Telstar AV-100, Spain
- Analytical balance: Precisa 205A SCS, Switzerland
- Vortex mixer: Velp Scientifica, Italy
- Microscope: Nikon Eclipse E200, Japan
- Automatic pipettes: Eppendorf, Germany; Gilson, France
- Culture media (blood agar, MacConkey agar, chocolate agar): Becton Dickinson, USA
- Aerobic and anaerobic blood culture bottles: BacT/ALERT, bioMérieux, France
- Anaerobic jar and Gas-Pak system: AnaeroPack, Mitsubishi Gas Chemical Company, Japan
- Automated blood culture system: BacT/ALERT 3D, bioMérieux, France
- Automated bacterial identification system: Phoenix 100, Becton Dickinson, USA
- Nephelometer: Phoenix Spec, Becton Dickinson, USA
- ELISA (Enzyme-linked immunosorbent assay) microplate washer: BioTek Elx50, BioTek Instruments, USA

• ELISA microplate reader: BioTek Elx800, BioTek Instruments, USA

• TNF-α rat ELISA kit: Cloud Clone (USCN), USA

• IL-6 rat ELISA kit: Cloud Clone (USCN), USA

• Hypochlorous acid (HOCl): CRYSTALIN®, 200 ppm HOCl, pH: 7.38, Türkiye

• Physiological saline solution: 0.9% Isotonic Sodium Chloride, POLİFLEKS®, Türkiye

• Ketamine: Ketasol 10%, 10 mL

• Xylazine: Rompun 2%, 25 mL

• 3/0 silk suture material: Betatech

• 3/0 prolene suture material: Betatech

This list provides a clear and concise description of the materials and devices used in the study, specifying their origins and manufacturers where applicable.

Surgical Procedures

Intraperitoneal anesthesia was administered to all four groups using 90 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride.

In the Sham group, only the abdomen was opened with a 2.5 cm midline incision. After 5 minutes, the incision was closed with sutures.

In the Control group, CLP + serum physiologic group, and CLP + hypochlorous acid group, the abdomen was opened with a 2.5 cm midline incision to expose the cecum. The cecum was removed from the abdomen without disrupting tissue perfusion. It was ligated with a 3/0 silk suture just below the ileocecal valve. The ligated segment was punctured once using an 18-gauge standard syringe tip, and a small amount of fecal matter was squeezed out. The cecum was then returned to the abdominal cavity, and the incision was closed with sutures.

The rats in the CLP + serum physiologic group and the CLP + hypochlorous acid group were euthanized 8 hours after the initial surgery. This was done by reopening the abdomen and excising the cecum distal to the ligation site. In one group, the abdominal cavity was lavaged three times with 5 mL of saline. In the other group, after three lavages with 5 mL of saline, the abdomen was additionally lavaged with 5 mL of hypochlorous acid for 2 minutes.

At the 24-hour mark, under the same conditions and maintaining the same sterility standards, a relaparotomy was performed. Blood samples were collected from the inferior vena cava of all animals, after which they were euthanized by cervical dislocation. Liver, spleen, and mesenteric lymph node tissue samples were then collected and placed in sterile containers for culture studies.

Blood samples obtained from the rats were cultured in aerobic and anaerobic blood culture bottles (BacT/ALERT, bioMérieux, France) for blood culture studies. Serum was separated by centrifugation for the determination of cytokine levels (TNF-α and IL-6) using enzyme-linked immunosorbent assay. The samples were stored at -80°C until the day of analysis.

Study of Aerobic and Anaerobic Blood Cultures

Blood cultures were monitored for five days using the BacT/ALERT 3D automated blood culture system (bioMérieux, France). Samples from blood culture bottles showing positive growth signals were gram-stained and inoculated onto blood agar, MacConkey agar, and chocolate agar (Becton Dickinson, USA). They were then incubated for 24-48 hours at 37°C under aerobic and anaerobic conditions (AnaeroPack, Mitsubishi Gas Chemical Company, Japan).

Study of Liver, Spleen, and Mesenteric Lymph Node Tissue Cultures

Tissue samples (liver, spleen, and mesenteric lymph nodes [MLN]) were homogenized by vortexing and then quantitatively inoculated onto blood agar, MacConkey agar, and chocolate agar (Becton Dickinson, USA). They were then incubated for 24-48 hours at 37°C under both aerobic and anaerobic conditions (AnaeroPack, Mitsubishi Gas Chemical Company, Japan). Bacterial colonization was assessed by counting colony-forming units (CFU) per gram of tissue homogenate. Identification of isolated strains at the species level was performed using the Phoenix 100 automated system (Becton Dickinson, USA), in addition to conventional microbiological methods.

Determination of Cytokine Levels

Serum samples were previously centrifuged, prepared, and stored at -80°C until the day of analysis. They were thawed and homogenized several times before the study. The absorbance values (optical density) for TNF-α (Rat TNF-α ELISA kit, Rel Assay Diagnostics, Türkiye) and IL-6 (Rat IL-6 ELISA kit, Rel Assay Diagnostics, Türkiye) in the rat sera were measured spectrophotometrically at 450 nm using an ELISA microplate reader (BioTek Elx800, BioTek Instruments, USA). With the help of a standard curve, the levels of TNF-α and IL-6 corresponding to the absorbance values in serum samples were determined. The results were expressed as pg/mL for TNF-α and ng/L for IL-6.

Ethical Statement

Permission from the Animal Experiments Ethics Committee of Süleyman Demirel University (SDU), dated 23.09.2021 and numbered 09/04, was obtained for this study. All surgical pro-

cedures were performed at the SDU Experimental Animal and Medical Research and Application Center (DEHATAM). Other experimental procedures were carried out in the laboratories of the Department of Microbiology, Faculty of Medicine, SDU. This study was supported by the SDU Scientific Research Coordination Unit under project number TTU-2021-8432. The study was conducted in accordance with the Declaration of Helsinki.

Statistical Analysis

The data were transferred to IBM SPSS version 23 (IBM Inc., Chicago, IL, USA) and analyzed using statistical methods. Relationships between categorical variables were analyzed using the Chi-square test. The normality of continuous variables was assessed with the Kolmogorov-Smirnov test, and homogeneity of variance was evaluated using Levene's test. The Mann-Whitney U test was used for two-group comparisons when normal distribution was not observed. In all analyses, a p value of <0.05 was considered statistically significant.

RESULTS

The study included 11 rats in each of the Sham, Control, CLP+HA, and CLP+SP groups, totaling 44 rats. One rat in the Control group was excluded from the study due to death (EX); the cause of death is unknown.

Liver Tissue Culture

Analysis of liver tissue cultures revealed no bacterial growth in 22 rats (51.2%). Escherichia coli growth was observed in 18 rats (41.9%), while growth of both Escherichia coli and Proteus mirabilis was detected in three rats (7%).

Liver tissue culture growth was detected in one rat in the Sham group, nine rats in the Control group, seven rats in the CLP+SP group, and one rat in the CLP+HA group. Escherichia coli growth in liver tissue culture was observed in 90% of the Control group, 9.1% of the Sham group, 63.6% of the CLP+SP group, and 9.1% of the CLP+HA group. Escherichia coli + Proteus mirabilis growth was observed only in 27.3% of the rats in the CLP+SP group (Table 1). A statistically significant difference in liver tissue culture was observed between rats in the Sham and Control groups (p<0.001). A statistically significant difference was also found between rats in the Sham and CLP+SP groups (p<0.001). However, no statistically significant difference was observed between rats in the Sham

Table 1. Liver tissue culture growth by rat group

	Sham (n=11)	Control (n=10)	CLP+SP (n=11)	CLP+HA (n=11)
Liver Tissue Culture		**n (%)**		
Dominant Species				
E. coli	1 (9.1)	9 (90.0)	7 (63.6)	1 (9.1)
E. coli + P. mirabilis	0 (0.0)	0 (0.0)	3 (27.3)	0 (0.0)
Reproduction detected	1	9	10	1

Chi-square test.

Table 2. Spleen tissue culture growth by rat group

	Sham (n=11)	Control (n=10)	CLP+SP (n=11)	CLP+HA (n=11)
Spleen Tissue Culture		**n (%)**		
Dominant Species				
E. coli	1 (9.1)	7 (70.0)	6 (54.5)	1 (9.1)
E. coli + E. faecalis	0 (0.0)	0 (0.0)	1 (9.1)	0 (0.0)
E. coli + P. mirabilis	0 (0.0)	1 (10.0)	2 (18.2)	0 (0.0)
Reproduction detected	1	8	9	1

Chi-square test.

Table 3. Mesenteric lymph node tissue culture growth by rat groups

	Sham (n=11)	Control (n=10)	CLP+SP (n=11)	CLP+HA (n=11)
Mesenteric lymph node tissue culture		**n (%)**		
Dominant Species				
E. coli	2 (18.2)	8 (80.0)	9 (81.8)	3 (27.3)
E. coli + E. faecalis	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)
E. coli + E. cloacae	1 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)
E. coli + P. mirabilis	0 (0.0)	2 (20.0)	2 (18.2)	0 (0.0)
Reproduction detected	3	10	11	4

Chi-square test.

and CLP+HA groups (p=0.999). Similarly, there was no statistically significant difference between the Control and CLP+SF groups (p=0.214). In contrast, a statistically significant difference was found between rats in the Control and CLP+HA groups (p<0.001), as well as between rats in the CLP+SP and CLP+HA groups (p<0.001) (Table 7).

Spleen Tissue Culture

According to spleen tissue culture results, no bacterial growth was observed in 24 rats (55.8%), while 15 rats (34.9%) showed growth of Escherichia coli. Growth of Escherichia coli and Enterococcus faecalis was detected in one rat (2.3%), and growth of Escherichia coli and Proteus mirabilis was detected in three rats (7%).

Among the rats whose spleen tissue cultures were examined, Escherichia coli growth was observed in 70% of the Control group, 9.1% of the Sham group, 54.5% of the CLP+SP group, and 9.1% of the CLP+HA group. Escherichia coli + Enterococcus faecalis growth was detected only in 9.1% of rats in the CLP+SP group, while Escherichia coli + Proteus mirabilis growth was detected in 18.2% of rats in the CLP+SP group (Table 2). A statistically significant difference in spleen tissue culture was observed between rats in the Sham and Control groups (p=0.003). There was also a statistically significant difference in spleen tissue culture between rats in the Sham and CLP+SP groups (p=0.004). No statistically significant difference in spleen tissue culture was found between rats in the Sham and CLP+HA groups (p=0.999), or between rats in the Control and CLP+SP groups (p=0.999). However, a statistically significant difference in spleen tissue culture was observed between rats in the Control and CLP+HA groups (p=0.003), and between rats in the CLP+SP and CLP+HA groups (p=0.004) (Table 7).

Mesenteric Lymph Node Tissue Culture

When mesenteric lymph node tissue cultures of rats were analyzed, 15 rats (34.9%) showed no bacterial growth. Escherichia coli was detected in 22 rats (51.2%), Escherichia coli + Proteus mirabilis in four rats (9.3%), Escherichia coli + Enterococcus faecalis in one rat (2.3%), and Escherichia coli + Enterobacter cloacae in one rat (2.3%).

According to the mesenteric lymph tissue culture results, Escherichia coli was grown in 80% of the Control group, 18.2% of the Sham group, 81.8% of the CLP+SP group, and 27.3% of the CLP+HA group. Escherichia coli + Enterococcus faecalis growth was seen only in 9.1% of the CLP+HA group; Escherichia coli + Enterobacter cloacae was observed in 9.1% of the Sham group; and Escherichia coli + Proteus mirabilis was found in 20% of the Control group and 18.2% of the CLP+SP group (Table 3). A statistically significant difference was observed between rats in the Sham and Control groups (p<0.001), and between the Sham and CLP+SP groups (p<0.001). No statistically significant difference was found between the Sham and CLP+HA groups (p=0.999), or between the Control and CLP+SP groups (p=0.999). However, statistically significant differences were found between the Control and CLP+HA groups (p=0.002), and between the CLP+SP and CLP+HA groups (p=0.001) (Table 7).

Blood Culture

According to the blood cultures of the rats, 32 (74.4%) showed no bacterial growth. Escherichia coli was grown in six rats (14.0%), Escherichia coli and Enterococcus faecalis in two rats (4.7%), Escherichia coli and Proteus mirabilis in two rats (4.7%), and Escherichia coli and Staphylococcus aureus in one rat (2.3%).

When blood cultures were analyzed by group, Escherichia coli growth was observed in 20% of the Control group, 27.3% of the CLP+SP group, and 9.1% of the CLP+HA group. Escherichia coli + Enterococcus faecalis growth was seen in 9.1% of the Sham group and 9.1% of the CLP+HA group. Escherichia coli + Staphylococcus aureus growth was detected in 10% of the Control group, and Escherichia coli + Proteus mira-

Table 4. Blood culture growth by rat group

	Sham (n=11)	Control (n=10)	CLP+SP (n=11)	CLP+HA (n=11)
Blood Culture		**n (%)**		
Dominant Species				
E. coli	0 (0.0)	2 (20.0)	3 (27.3)	1 (9.1)
E. coli + E. faecalis	1 (9.1)	0 (0.0)	0 (0.0)	1 (9.1)
E. coli + S. aureus	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)
E. coli + P. mirabilis	0 (0.0)	0 (0.0)	2 (18.2)	0 (0.0)
Reproduction detected	1	3	5	2

Chi-square test.

Table 5. Enzyme-linked immunosorbent assay (ELISA) values by rat group

	Sham (n=11)	Control (n=10)	CLP+SP (n=11)	CLP+HA (n=11)
		Median±Standard Deviation		
TNF-α (pg/mL)	37.9±11.2	43.7±7.5	44.3±8.4	43.5±14.9

Chi-square test.

Table 6. Interleukin-6 (IL-6) levels by rat group

	Sham (n=11)	Control (n=10)	CLP+SP (n=11)	CLP+HA (n=11)
		Median±Standard Deviation		
IL-6 (pg/mL)	24.4±12.5	27.7±9.3	31.2±7.8	27.3±17.5

Mann-Whitney U test

Table 7. Statistical comparison of enzyme-linked immunosorbent assay (ELISA) and culture results between groups

	Sham vs. Control	Sham vs. CLP+SP	Sham vs. CLP+HA	Control vs. CLP+SP	Control vs. CLP+HA	CLP+SP vs. CLP+HA
Liver Culture	p<0.001	p<0.001	p=0.999	p=0.214	p<0.001	p<0.001
Spleen Culture	p=0.003	p=0.004	p=0.999	p=0.999	p=0.003	p=0.004
Mesenteric Lymph Node Culture	p<0.001	p<0.001	p=0.999	p=0.999	p=0.002	p=0.001
Blood Culture	p=0.201	p=0.035	p=0.999	p=0.635	p=0.672	p=0.181
TNF-α	p=0.622	p=0.531	p=0.641	p=0.999	p=0.999	p=0.998
IL-6	p=0.929	p=0.577	p=0.946	p=0.916	p=0.999	p=0.882

bilis was observed in 18.2% of the rats (Table 4). There was no statistically significant difference between the Sham and Control groups (p=0.201). A statistically significant difference was found between the Sham and CLP+SP groups (p=0.035). No statistically significant difference was observed between the Sham and CLP+HA groups (p=0.999), the Control and CLP+SP groups (p=0.635), the Control and CLP+HA groups (p=0.672), or the CLP+SP and CLP+HA groups (p=0.181) (Table 7).

ELISA Results

The mean TNF-α value of the rats was 42.31±10.89 pg/mL, with a minimum value of 25.37 and a maximum value of 76.77. The minimum IL-6 value was 7.80, the maximum was 65.26, and the mean IL-6 value was 27.68±12.22 pg/mL. The mean TNF-α value was 43.7±7.5 pg/mL in the Control group, 37.9±11.2 pg/mL in the Sham group, 44.3±8.4 pg/mL in the CLP+SP group, and 43.5±14.9 pg/mL in the CLP+HA group (Table 5). The mean TNF-α value did not show a statistically significant difference between the groups (p>0.05). Specifically, there were no statistically significant differences in mean TNF-α levels between the Sham and Control, Sham and CLP+SP, Sham and CLP+HA, Control and CLP+SP, Control and CLP+HA, or CLP+SP and CLP+HA groups (p=0.622, p=0.531, p=0.641, p=0.999, p=0.999, and p=0.998, respectively) (Table 7).

The mean IL-6 value was 27.7±9.3 pg/mL in the Control group, 24.4±12.5 pg/mL in the Sham group, 31.2±7.8 pg/mL in the CLP+SP group, and 27.3±17.5 pg/mL in the CLP+HA group (Table 6). There were no statistically significant differences in mean IL-6 values between the Sham and Control, Sham and CLP+SP, Sham and CLP+HA, Control and CLP+SP, Control and CLP+HA, or CLP+SP and CLP+HA groups (p=0.929, p=0.577, p=0.946, p=0.916, p=0.999, and p=0.882, respectively) (Table 7).

DISCUSSION

A review of the literature reveals that numerous experimental models have been developed for studying peritonitis and intra-abdominal sepsis. Common intra-abdominal approaches include the intraperitoneal administration of lipopolysaccharide, direct intraperitoneal inoculation of live bacteria, and the cecal ligation and puncture technique.[11]

The CLP model offers several advantages as an experimental approach to peritonitis. It is simpler and more cost-effective than models requiring lipopolysaccharides or live microorganisms, which can be expensive and difficult to obtain. Moreover, the CLP model closely replicates clinical conditions commonly seen in hospitals, such as perforated appendicitis, diverticulitis, and colon perforation. Unlike other experimental peritonitis and intra-abdominal sepsis models that involve specific microorganisms, the CLP model involves polymicrobial transmission.[1,12]

In this study, the most notable finding was that hypochlorous acid significantly suppressed bacterial translocation in liver, spleen, and mesenteric lymph node tissue cultures in a rat model of experimental peritonitis induced by cecal ligation and puncture. Importantly, this antibacterial effect occurred without significantly reducing systemic inflammatory markers such as TNF-α and IL-6.

Although bacterial translocation was significantly reduced in tissue cultures, the absence of a statistically significant difference in blood culture results, despite a numerical decrease, suggests that bacterial presence may still occur at levels below the detection threshold of culture methods. It should be noted that in microbiological culture studies, bacterial growth may not always be observed, even when bacteria are present in the tissues. This may be due to limitations related to sample handling, bacterial viability, or the technical sensitivity of the culture methods. Therefore, findings should be interpreted with caution, taking into account the possibility of false-negative culture results.

Our results align with previous literature supporting the antibacterial efficacy of HOCl. Several studies have demonstrated that HOCl can significantly reduce bacterial contamination and promote wound healing without causing cytotoxic effects.

In the study by Ross JT et al., IL-6 or TNF-α are identified as early immune responses to tissue damage and/or infection. These cytokines are responsible for fever induction, activation of endothelial cells, and recruitment of polymorphonuclear cells, such as neutrophils, to the site of inflammation. They are among the most important mediators in the pathophysiology of sepsis; however, their levels also increase in non-infectious inflammatory conditions. Their lack of specificity, short plasma half-life, and low biological stability are key limitations when using these cytokines in studies.[2]

In a study by Frazier WJ et al.,[3] TNF-α release persists alongside the activation of immune cells and the secretion of pro-inflammatory cytokines, which promote cellular migration to the vascular endothelium. As this process becomes systemic, the symptoms intensify with the progression of inflammation.[4]

In our study, we found that the mean values of TNF-α and IL-6, both inflammatory markers measured by ELISA from blood samples, did not differ significantly between the experimental rat groups. This indicates that HOCl did not suppress inflammation.

HOCl is used in many fields due to its broad antibacterial, antiviral, antiseptic, and disinfectant properties. Although it was discovered many years ago, its use was limited because of its instability and its pH being outside physiological ranges.

A study by Davis SC et al.[8] evaluated the efficacy of a hypochlorous acid-containing wound management solution (WMS) compared to sterile saline on methicillin-resistant

Staphylococcus aureus and wound healing in mouse models. The authors found that topical WMS combined with debridement significantly reduced methicillin-resistant Staphylococcus aureus contamination.[11]

In a prospective study by Ricci E et al.,[13] it was demonstrated that HOCl solution could be effectively used to prepare the wound bed during the healing process in patients with hard-to-heal ulcers of various etiologies. In a study by Hasan S et al.,[14] irrigation of breast lumps with HOCl during revision aesthetic breast surgery reduced the recurrence of capsular contracture and lowered the incidence of infectious complications. No allergic reactions or side effects were observed.

Anagnostopoulos AG et al.[15] compared the in vitro efficacy of 0.01% HOCl, 5% povidone-iodine, 4% chlorhexidine gluconate, and 70% isopropyl alcohol against common skin microorganisms. HOCl was found to have antiseptic properties equal to or greater than those of the other antiseptics tested.

Romanowski EG et al.[16] showed that 0.01% HOCl did not disrupt the biofilm structures formed by Staphylococcus aureus and coagulase-negative streptococci in cases of blepharitis, but it was effective in eliminating the bacteria at the desired level.

Burian EA et al.[17] compared HOCl solution with sterile 0.9% sodium chloride solution in the treatment of acute wounds. Twenty healthy volunteers were divided into two groups, and open wounds were created on the forearm. The wounds were irrigated on days 0, 2, and 4 with HOCl solution in one group and sterile 0.9% sodium chloride solution in the other. Re-epithelialization, bacterial growth, and colony counts in swab cultures were compared. The study demonstrated that irrigation with stable HOCl solution provided a faster and more lasting antimicrobial effect, a 14% increase in epithelialization, and overall improvement in acute wound healing compared to 0.9% sodium chloride solution.

In a study by Gözüküçük, A. et al.,[18] wound healing and antibacterial efficacy of HOCl and povidone-iodine, used as disinfectants prior to circumcision, were compared. The study concluded that the use of HOCl as a disinfectant before circumcision was safe.

In a study conducted by Jiang RS et al.[19] to investigate the efficacy of HOCl nasal spray as an adjuvant treatment following functional endoscopic sinus surgery, it was shown that HOCl had similar efficacy to routine nasal irrigation solutions and could be used as an alternative.

In a study by Day A et al.[20] investigating the effectiveness of methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa biofilms, it was reported that HOCl-based wound cleanser solutions were similarly effective in eliminating bacteria compared to collagen-based wound cleansers, but with lower cytotoxicity.

In a 2021 study conducted by Ball RL et al.[21] on rats, HOCl and normal saline were compared for safety in intracavitary irrigation. The study reported that HOCl was safe for intrathecal, intrathoracic, and intraperitoneal irrigation in rats undergoing laminectomy, thoracotomy, and laparotomy. However, the authors noted that further studies are needed to assess its efficacy in infected areas.

Foster KN et al.[22] compared HOCl with 5% sulfamylon as postoperative dressings in burn patients requiring skin grafting. The study demonstrated equivalent efficacy and safety for both treatments, although HOCl was found to be more cost-effective.

In our study, we compared saline, commonly used for intra-abdominal irrigation, with HOCl, which we believe has the potential for future use in clinical practice.

The lack of statistically significant difference between the CLP+HA group and the other groups in blood culture results, despite a numerical decrease in bacterial growth, is likely due to limitations such as the small sample size and the short duration of the experiment inherent to the peritonitis-sepsis model.

However, in liver tissue culture, spleen tissue culture, and mesenteric lymph node tissue culture, a statistically significant difference was found between the CLP+HA group and the Control group, which we considered representative of an intra-abdominal "dirty" environment. Similarly, a statistically significant difference was observed between the CLP+HA group and the CLP+SP group, in which intra-abdominal lavage was performed using saline, a solution routinely employed in surgical practice.

Another consideration is the generalizability of our results. Although the CLP model closely mimics clinical intra-abdominal sepsis conditions such as perforated appendicitis and diverticulitis, differences between rat models and human physiology must be acknowledged. Variations in immune response, bacterial flora, and wound healing dynamics across species may limit the direct extrapolation of these findings to human clinical practice. Nonetheless, our results provide promising preliminary evidence that intraperitoneal HOCl lavage may have clinical value in reducing bacterial translocation and controlling intra-abdominal infection.

From a future research perspective, our findings suggest that HOCl could serve as an effective adjunctive therapy for intra-abdominal infections and sepsis. Given its broad-spectrum antimicrobial properties, low toxicity, and ability to reduce bacterial translocation without impairing systemic immune responses, further clinical trials are warranted to assess its efficacy in human subjects. Investigations into optimal dosing, timing, and comparative effectiveness against standard treatments (e.g., saline irrigation) could offer valuable insights for clinical application.

This study has several strengths. Notably, it is one of the few experimental investigations specifically evaluating the

impact of HOCl on bacterial translocation in an established model of intra-abdominal sepsis. The use of multiple tissue cultures (liver, spleen, mesenteric lymph nodes) provided a comprehensive assessment of bacterial dissemination. However, there are important limitations that should be acknowledged. The relatively small sample size and short experimental follow-up period may have limited the ability to detect differences in systemic outcomes, such as blood culture positivity and cytokine levels. Moreover, the inherent limitations of culture methods, including the potential for false-negative results, may have influenced the findings. Despite these limitations, the reproducibility and clinical relevance of the CLP model, combined with the consistency of tissue culture results, strengthen the validity of our conclusions.

CONCLUSION

In this study, we found that HOCl significantly reduces bacterial translocation without suppressing inflammation. We anticipate that its broader use in clinical practice could help reduce mortality and morbidity associated with intra-abdominal sepsis, shorten hospitalization in patients with perforation-induced peritonitis/intra-abdominal sepsis, lower the medical costs related to sepsis and systemic inflammatory response syndrome, and ultimately contribute to national healthcare savings. In light of these findings, investigating HOCl in the context of bacterial translocation in experimental peritonitis models may guide future research. Further studies are needed to confirm these results in larger animal models and clinical trials.

Ethics Committee Approval: This study was approved by the Animal Experiments Ethics Committee of Süleyman Demirel University (Date: 23.09.2021, Decision No: 09/04).

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DENEYSEL ÇALIŞMA - *ÖZ*

Sıçanlarda oluşturulan deneysel peritonit modelinde intraperitoneal hipokloröz asitin (HOCl) bakteriyel translokasyona etkisi

AMAÇ: Bu çalışmada özellikle karın içi irrigasyonda rutin olarak kullandığımız serum fizyolojik ile gelecekte klinik pratikte kullanılmasının uygun olacağını düşündüğümüz HOCl'yi sıçanlarda oluşturulan peritonit modelinde bakteriyel translokasyona etkisini karşılaştırdık.
GEREÇ VE YÖNTEM: Dört grup oluşturuldu. Sham, kontrol, CLP+SF, CLP+HA gruplarında 11'er sıçan dahil ederek toplamda 44 sıçan ile çalışıldı. Kontrol grubundaki 1 sıçan öldüğü için çalışma dışı bırakıldı. Çalışmada; özellikle karın içi irrigasyonda rutin olarak kullandığımız serum fizyolojik ile gelecekte klinik pratikte kullanılmasının uygun olacağını düşündüğümüz HOCl karşılaştırıldı.
BULGULAR: CLP+SF ile CLP+HA grubundaki sıçanlar arasında karaciğer doku kültürü, dalak doku kültürü, mezenter lenf nodu doku kültürü bakımından istatistiksel olarak anlamlı bir farklılık olduğu gözlendi (sırasıyla, p<0.001, p=0.004, p=0.001). Ancak CLP+SF ile CLP+HA grubundaki sıçanlar arasında kan kültürü bakımında anlamlı bir fark olmadığı tespit edildi (p=0.181). Kan kültüründe sayısal olarak bakteri üremesini azaltmasına rağmen CLP+HA grubuyla diğer gruplar arasında istatistiksel olarak anlamlı bir ilişki olmadığının saptanması örneklem büyüklüğünün kısıtlılığına ve deneyin peritonit/sepsis modeli olması nedeniyle deney süresinin kısalığına bağlandı. Bununla birlikte alınan kan örneklerinden çalışılan ELISA sonuçlarında inflamasyon belirteçleri olan TNF-alfa ve IL-6 ortalama değerlerinin deneydeki sıçan gruplarına göre istatistiksel olarak anlamlı ölçüde farklılık göstermediğini ve böylece HOCL'nin inflamasyonu baskılamadan bakteriyel translokasyonu belirgin düzeyde azalttığı bulundu.
SONUÇ: Klinik pratikte HOCl'nin kullanımının yaygınlaşmasıyla perforasyona sekonder peritonit/karın içi sepsiste mortalite ve morbiditenin düşeceği, hastanede yatış süresinin kısalacağı, artan medikal tedavi masraflarının düşeceği ve sağlık alanında ülke ekonomisine katlı sağlayacağı öngörülmektedir.

Anahtar sözcükler: Bakteriyal translokasyon; hipoklorik asit, IL-6; peritonit; TNF- alfa.

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