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Research Article

Evaluation of Effectiveness of Neutral-pH Superoxidized Solution (NSOS) with Peritoneal Lavage in Rat Fecal Peritonitis Model: An Experimental Study

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Abstract

This experimental study aimed to evaluate the effectiveness of NSOS (Neutral-pH superoxidized solution) with peritoneal lavage in rat fecal peritonitis models. Forty Wistar rats weighing between 250-300 g were used for the study. All rats were inducted for fecal peritonitis formation. The rats were divided into five groups as follows; Group 0: control, no intervention. Group 1: Application of 10 mg/kg NSOS into the peritoneal cavity after 6 h of induction of peritonitis. Group 2: 10 mg/kg NSOS application and repeated at 24 and 48 h. Group 3: NSOS + Antibiotic treatment (Seftriakson 30 mg/kg/day IM 2x1 + metronidazol 15 mg/kg/day IM 2x1). Group 4: Only antibiotic treatment (Seftriakson 30 mg/kg/day IM 2x1 + metronidazol 15 mg/kg/day IM 2x1). The animals were examined for peritoneal and thoracal abscess formation, adherences, and any abnormality with inspection after sacrificing on the 7th day. The peritoneal lavage fluid culture for microbiological analysis and blood samples were taken for blood cultures, biochemical and infectious parameters of WBC, CRP, TNF- α, IL-6, IL-1 β, and IL-10. Peritonitis was developed in all rats at the end of follow-up. No death was observed in rats on the seventh day of the experiment. Group 3 (NSOS + Antibiotic treatment) showed the most significant improvement in the infection of peritoneal fluid. NSOS and antibiotic together (Group 3) were found to be more effective against *Klebsiella* than *Enterococcus* sp. The blood cultures showed a significant reduction in all groups. The infectious parameters including IL-6, IL-1 β, and IL-10 showed no significant difference in the first week of treatment between all groups. Only TNF- α was observed significantly lower in group 3 when compared to the other groups (P=0.001). Peritoneal lavage with neutral pH-superoxidized water plus an antibiotic regimen is the most effective treatment in the rat fecal peritonitis model. Further studies including human subjects are needed to investigate its effectiveness and validity.

Keywords: Fecal peritonitis, Neutral pH, NSOS, Rat, Antibiotic treatment

Introduction

Intraabdominal infections and sepsis are one of the leading causes of morbidity and mortality in patients with surgical interventions [1]. Peritonitis is usually caused by the bacterial contamination of the visceral peritoneum due to gastrointestinal organ perforations [2]. The infections in peritonitis usually come from the duodenum, stomach, gallbladder, pancreas, small intestine, colon, bladder, ovary, and renal system. Generalized peritonitis is an ominous sign of health status and requires prompt surgical therapy. If left untreated, the peritonitis can cause sepsis development quickly and sepsis is associated with a high rates of mortality and morbidity resulting from multiple organ failures [3].

Fecal peritonitis occurs due to the contamination of fecal material in the case of the bowel perforation. Enterococcus and Klebsiella are one of the most seen microorganisms in peritoneal infections and abscesses formation [4]. Surgical therapy is the main factor in the treatment of fecal peritonitis. During this surgery, the organ perforations are repaired and peritoneal lavage with normal saline irrigation is the most widely used method. In the literature, several studies showed the effect of different agents used for peritoneal lavage including ropivacaine, hydrogen-

rich saline, glycyrrhiza glabra cold atmospheric plasma, boric acid-linked ampicillin and lidocaine [5-11].

Neutral-pH superoxide water (NSOS) is formed by applying an electric current to salty water, followed by an electrochemical process in aqueous solutions from tap water and NaCI [12]. It has been used as a broad-spectrum disinfectant and shown to be non-toxic to human bodies. There are several studies reported that it has a significant antimicrobial effectiveness against bacteria and viruses [13]. The super-oxidized solutions have been used on ocular surfaces or wound infections and showed the confirmation of feasibility and safety in experimental studies [14].

In our previous experimental rat study on healthy peritoneal surfaces showed that pH-neutral superoxidized water does not result in any remarkable toxicity or complications [15]. Thus, we aimed to investigate its safety and effectiveness for peritoneal irrigation in the fecal peritonitis model.

Material and Methods

Ethical Approval

This study was approved by the Ethics Committee for Animal Experiments of Van Yüzüncü Yıl University, reference number 2020/10-11, dated 28.10.2020, and was performed in the Experimental Animal Research Laboratory of the university, located in Van, Türkiye.

Animals

Forty Wistar-albino rats were used in this experimental study. All rats were treated humanely in accordance with the Declaration of Helsinki. The animals were fed in accordance with the standards determined by the institution. The rats were randomly divided equally into 5 groups.

Group 0: Control group, fecal peritonitis was established and no other treatment was applied.

Group 1: *10 mg/kg NSOS applied into the peritoneal* cavity 6 h after the establishment of fecal peritonitis.

Group 2: 10 mg/kg NSOS applied into peritoneal cavity 6 h after establishment of fecal peritonitis and repeated at 24 and 48 h.

Group 3: 10 mg/kg NSOS and Antibiotic treatment (Seftriakson [Novasef®, Sanofi] 30 mg/kg/day IM 2x1 + metronidazol [Flagyl®, Eczacıbaşı] 15 mg/kg/day IM 2x1) applied into the peritoneal cavity 6 h after the establishment of fecal peritonitis.

Group 4: Only Antibiotic treatment (Seftriakson 30 mg/kg/day IM 2x1 + metronidazol 15 mg/kg/day IM 2x1) was applied 6 h after the establishment of fecal peritonitis.

Establishment of Fecal Peritonitis

Fecal peritonitis was created by the method defined by Buyne OR et al[16]. Fresh faeces from Wistar rats were obtained and suspended in twice the volume sterile water. An autoclave was used for sterilization of suspension. Then centrifugation process was performed and upper two layers were autclaved again and stored at 4°C. At the day of study 1 mL of fecal suspension was mixed with 0.5 mL of Schaedler solution including Escherichia coli bacillus level 10^4-10^8 cfu and B. fragilis level 10^4 cfu isolate which were obtained from the laboratory and then, 10 mL/kg of this fecal suspension was injected into the peritoneal cavity of the subjects. 6 h after injection of fecal material, the peritoneal cavity was irrigated with 10 mL/kg NSOS in groups 1, 2, and 3.

The rats were followed up daily for fever, tacyhpnea, weight, feeding status, and general appearance (flat hair, drowsiness, anxiety, blackness around the eyes). The rats were fed as Ad-Libitum. Single dose of 1 mg/kg of xylazine intraperitoneally administered after the operation for pain management. 24 h after the establishment of fecal peritonitis, 5-10 mL of saline was injected into the peritoneal cavity with massage and so distribution was created. 2-3 min after that procedure, 1-3 mL of peritoneal fluid was withdrawn. This fluid was kept for biochemical and microbiological analysis. Also, blood samples were taken for culture analysis.

There was no death in the subjects until Day 7. On the 7th day, the subjects were anesthetized with 50 mg/kg ketamine HCl (Ketalar®, Pfizer) and 10 mg/kg xylazine HCl (Rompun®, Bayer), and the abdominal and thorax cavities were opened, abscesses were investigated in the abdomen, and whether there was any adhesion was investigated. Fluid and tissue samples were taken for culture in the peritoneal cavity. Any abscess was investigated in the thoracic cavity. Blood samples were taken for biochemical analysis and culture, and then the animals were sacrificed. Among the biochemical parameters, CRP, TNF-α, IL-6, IL-1β, and IL-10 were evaluated by ELISA method. The hemogram was checked on an autoanalyzer.

Statistical Analysis

Descriptive statistics for continuous variables among the features emphasized expressed as Mean, Standard Deviation, Minimum and Maximum values, and also it is expressed as numbers and percentages for categorical variables. The Kruskal-Wallis test was used for comparisons made by groups in terms of continuous variables. The Chi-square test and multiple correspondence analysis were used to determine the relationship between groups and categorical variables. In the calculations, the statistical significance level was taken as 5% and the SPSS (ver:21) statistical package program was used for the calculations.

Results

All rats survived during the first week of the experiment after peritoneal lavages. The peritoneal infections developed in all groups. All groups were observed to have blood culture positivity at 24 h after the induction of peritonitis. Group 0, the control group, showed similar results on the first day and seventh days of the experiment. Although it was not statistically significant, group 1 showed a significant improvement of NSOS on the *Klebsiella* when compared the first day to seventh day. In group 2, the repeated use of NSOS did not show the additional effect on the *Klebsiella,* however, it showed some positive effect on the Enterococcus species *(Table 1)*.

Group 3 showed the most effectiveness and NSOS plus antibiotic regimen was the most effective treatment for *E. coli* and *Klebsiella*. This was statistically significant with p value of 0.016. First day of positive peritoneal culture's number was 17/24 and the seventh day was 4/24 (70% vs 16%, P=0.016). The peritoneal culture results showed that NSOS plus antibiotic regimens were the most effective method for the rat peritonitis model.

When analyzing the blood cultures, all groups had positive cultures for infection on first day of experiment. All groups showed a significant reduction for infections on the seventh day. Group 4 was observed no effectivity against *Enterococcus* species. However, the most significant improvement in the infections with regards to blood culture positivity taken for all three types of microorganisms *(Klebsiella, E. coli,* and *Enterococcus)* was observed in group 3 as 10/24 at first day vs 1/24 at seventh day with P=0.01) *(Table 2)*.

The peritoneal and blood cultures were taken at first day and seventh day of experiment. In total, 48 cultures were taken for each group. *Table 3* shows the comparison of Peritoneal Fluid and blood culture positivity *(Klebsiella, E. coli,* and *Enterococcus)* of first and seventh day of experiment in groups. All groups showed a decrease in culture positivity numbers. However, only group 3 (NSOS + antibiotic regimen) had a statistically significantly reduction in culture positivity (27/48, (56%) vs 5/48, (10%), P=0.022) *(Fig. 1)*.

The blood parameters including WBC, CRP, TNF-α, IL-6, IL-1β, IL-10 were analyzed at seventh day of experiment as shown in *Table 4*. It was observed that IL-6, IL-1β and IL-10 showed no significant difference in first week of treatment between all groups. TNF-α was observed significantly lower in group 3 when compared to the other

Table 1. *Peritoneal fluid cultures of groups at the 7th day*

Causative Bacteria		Group 0 (n:8) n (%)	Group 1 (n:8) n (%)	Group 2 (n:8) n (%)	Group 3 (n:8) n (%)	Group 4 (n:8) n (%)	P Value
Klebsiella	Positive Negative	4 (50%) 4 (50%)	2 (25%) 6 (75%)	5 (62%) 3 (37%)	1 (12%)[a] 7 (87%)	6 (75%) 2 (25%)	0.03
Enterococcus	Positive Negative	4 (50%) 4 (50%)	4 (50%) 4 (50%)	2 (25%) 6 (74%)	3 (37%) 5 (62%)	5 (62%) 3 (37%)	0.12
E. coli	Positive Negative	3 (37%) 5 (62%)	3 (37%) 5 (62%)	4 (50%) 4 (50%)	0[a] 8 (100%)	2 (25%) 6 (75%)	0.02
Total cultures	Positive Negative	11 (45%) 13 (55%)	9 (37%) 15 (62%)	11 (45%) 13 (55%)	4 (16%)[a] 20 (84%)	13 (55%) 11 (45%)	0.016

P<0.05 shows statistical significance

Table 2. *Blood Cultures of groups at the 7th day*

Causative Bacteria		Group 0 (n:8) n (%)	Group 1 (n:8) n (%)	Group 2 (n:8) n (%)	Group 3 (n:8) n (%)	Group 4 (n:8) n (%)	P Value
Klebsiella	Positive Negative	2 (25%) 6 (75%)	1 (12.5%) 7 (87.5%)	1 (12.5%) 7 (87.5%)	0 [a] 8 (100%)	2 (25%) 6 (75%)	0.002
Enterococcus	Positive Negative	3(37.5%) 5(62.5%)	2 (25%) 6 (75%)	2 (25%) 6 (75%)	0 [a] 8 (100%)	5 (62.5%) 3 (37.5%)	0.032
E. coli	Positive Negative	2(25%) 6(75%)	3 (37.5%) 5 (62.5%)	2 (25%) 6 (75%)	1 (12.5%) 7 (87.5%)	2 (25%) 6 (75%)	0.082
Total cultures	Positive Negative	7(29%) 17(70%)	6 (25%) 18 (75%)	5 (20%) 19 (79%)	1 (4%)[a] 23 (96%)	9 (37.5%) 15 (62.5%)	0.01

P<0.05 shows statistical significance, a indicates the statistically significant difference

Table 3. *Comparison of total number of peritoneal Fluid and blood culture positivity (Klebsiella, E. coli, and Enteroccocus) of first and seventh day of experiment in groups*

Groups	First Day n (%)	Seventh Day n (%)	P Value
Group 0	22/48 (45%)	18/48 (37%)	0.794
Group 1	24/48 (50%)	15/48 (31%)	0.114
Group 2	24/48 (50%)	16/48 (33%)	0.446
Group 3	27/48 (56%)	5/48 (10%)	0.022
Group 4	23/48 (47%)	20/48 (41%)	0.999

P<0.05 shows statistical significance

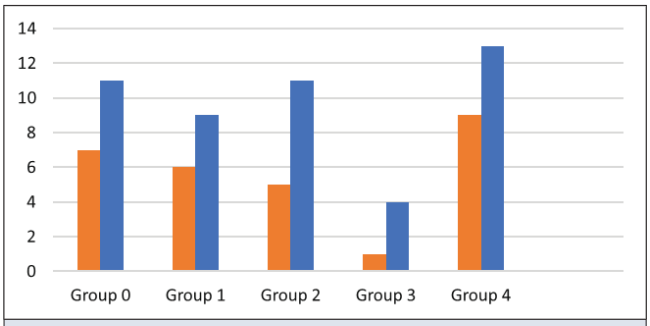

Fig 1. Total number of blood and peritoneal cultures positivity *(Klebsiella, E. coli,* and *Enterococcus)*

Table 4. *Comparison of biochemical-infectious-blood parameters in groups at the 7th day*

Parameters	Group 0 (n:8)	Group 1 (n:8)	Group 2 (n:8)	Group 3 (n:8)	Group 4 (n:8)	P Value
WBC, x10^3	11.09±1.55	8.20±3.81	7.32±1.04	6.68±0.84[a]	6.62±1.15[a]	0.002
CRP, mg/L	12.83 ±0.61	12.72±1.57	13.01±0.88	13.76±0.37	13.26±0.75	0.082
TNF-alpha, ng/L	125.27±23.54	131.04±22.78	127.86±20.61	98.53±17.52	125.89±9.34	0.082
IL-1, ng/mL	4.95±0.57	5.40±0.95	4.86±0.46	4.97±0.88	4.78±0.29	0.625
IL-6, ng/L	2.35±1.76	2.26±0.86	1.89±0.49	2.33±1.38	1.66±0.20	0.516
IL-10, pg/mL	44.11±0.41	43.85±0.13	44.00±0.45	43.72±0.03	43.76±0.10	0.121

P<0.05 shows statistical significance, WBC: White blood cell, CRP: C-reactive protein, TNF: Tumor necrosis factor, IL: Interleukin The measurements are shown as mean ± Standard deviation, a showed statistical difference

groups (98.5 3±17.52 ng/L, P=0.001). WBC was found to be significantly higher in Group 0, control group when compared to the other groups (11.09±1.55 x 10^3, P=0.002).

Discussion

Our previous animal study showed that pH-neutral SOS(NSOS) does not result in any significant toxicity or complications on the liver and peritoneal surfaces [15]. Moreover, in this study, we examined its effect on the infectious parameters in the fecal rat peritonitis model. This study showed that washing the peritoneal cavity with an NSOS plus antibiotic regimen has significant effectiveness in the treatment of rat fecal peritonitis model. Intraabdominal infections and abscess formations are one of the main reasons for morbidity and mortality after perforated appendicitis, tuba ovarian infections, or other peritoneal diseases [17]. Peritoneal irrigation is a challenging issue in the field of surgery and normal saline is traditionally a mostly used agent for peritoneal washings, however, this saline has no microbicidal activity. In the literature, there are many agents like lidocaine, ropivacaine, meropenem and moxifloxacin used to t reat this infection in rat peritonitis and also human subjects [6,7,18,19].

The peritoneum has a unique feature for its ability to deal with infections. The main mechanisms that have bactericidal activity are clearance from the diaphragmatic lymph system, phagocytization by host White blood cells and walled-off infection between the omentum and viscera [4,20]. These three factors can prevent intra-

abdominal infections at a certain level of bacterial load, however, the fecal contamination of the abdominal cavity in which a high load of microorganisms is encountered resulted in fail of defense systems. This eventually may lead to the development of sepsis and organ failures. Therefore studies have addressed the evaluation of effectiveness of different agents used for irrigation of peritoneal cavity during surgery. Aminoglycosides are the most used agent for peritoneal irrigation in cases of perforated appendicitis. A study by Shuaib et al.[21] evaluated the effectiveness of intraperitoneal gentamicin and clindamycin with peritoneal lavage in rat fecal peritonitis model and concluded that antibiotic peritoneal lavage alone reduces mortality rate when compared to a normal saline peritoneal lavage. Although the use of intraperitoneal antibiotics is thought to be safe and effective, studies focused on their bioavailability, long-term side effects, and cost-effectiveness.

Superoxidized water is a broad-spectrum antimicrobial and has been used in burn wounds, mediastinitis and open heart surgeries. Kubato et al.[12] studied the effect of strong acid electrolyzed water which is the older form of superoxide solutions on the surgical site infection of patients with perforated appendicitis and they found that peritoneal lavage and wound washing with superozidized water has no adverse effects and are effective for preventing surgical site infection. However, they used a strong acid agent that may be corrosive in mucoal surfaces. Therefore, NSOS which is used in our experimental study is a pH-neutral superoxidized water and formed by applying an electric current to salty water, followed by electro-chemical processed in aqueous solutions from pure water and sodium chloride. It is widely used as broad spectrum disinfectant and is non-toxic to human tissues unlike to the first supeoxide solutions which have low-pH values and corrosive features [22].

Landa-Solis et al.[23] studied the NSOS and reported that it has good antimicrobial activity, with significant advantages over strong acidic superozidized solutions, including neutral pH, decreased free active chlorine and a long shelf life. Our study confirmed this results that the Group 3 in which combined regimen of NSOS plus antibiotics showed the most significant improvement in the infectious state of rat peritonitis model. To date, this is the first randomized experimetal study that evaluated the effect of NSOS plus antibiotic regimen on rat peritonitis model. The results are promising that by applying and adding this non-toxic anti-bacterial solution to the intraperitoneal antibiotic lavage may prevent infections more effectively than other strategies.

It was worth noting that the standard treatment of peritonitis with antibiotics may be improved by adding the NSOS which is shown to be safe and non-toxic to human bodies and found to be effective when given with seftriakson-metronidazole combination together. Another point should be noted that our previous study is validated from the aspect of effectiveness, however, further clinical studies on human subjects is mandatory to implement its clinical use [15].

Some limitations should be declared about this study. Firtsly, it has a short term follow-up time of one week. Because some toxic effects or healing processes can be seen in a long-term time frame, this short time can be a weakness of our study. Secondly, the response to treatment was not measured by an objective method like bacteria colony number counting. Instead of it, the culture positivity was used. The main strength of this study is that all the procedures were performed by the same surgeon and culture analyses were done in the same hospital's laboratory.

In conclusion, this study's goal was to determine the effectiveness of NSOS in combination with antibiotics in the treatment of rat peritonitis model. Our findings revealed that peritoneal irrigation with NSOS plus an antibiotic regimen could improve the infectious state and survival of rats with peritonitis. Further studies including human subjects are needed to clarify our results and to determine its use in clinical practice.

Declarations

Availability of Data and Materials: The data used in this article will be provided by the corresponding author (A. Aras) upon request.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Author Contributions: Forming the hypothesis and planning the study: AA; Carrying out the experimental phase: AA, EK, SÇ, HS; Obtaining data and writing the article: AA, EK.

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