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Transfer factor treatment in management of peritonitis condition: An experimental study in rat

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ABSTRACT

Background: There is a role for the immune system in improving the outcome of peritonitis cases in children. Transfer factors are one immunomodulatory treatment that can increase the activity of natural killer (NK) cells to produce interferon-gamma (IFN-γ), which is thought to increase the phagocytic activity of macrophages. This study analyzed the effects of transfer factors on the phagocytic activity of macrophages in the intraperitoneal fluid of a Wistar rat model of peritonitis.

Methods: This experimental study had a post-test-only control group design and was carried out at the Laboratory of Pharmacology and Microbiology of Padjadjaran University, Bandung, Indonesia. It analyzed the effect of transfer factors on the phagocytic activity of macrophages in the intraperitoneal fluid of Wistar rats experiencing peritonitis after being injected with *Escherichia coli*. An unpaired comparative *t*-test was performed using the SPSS program to analyze the difference between transfer factor administration and macrophage phagocytic activity levels.

Results: There was a statistically significant difference between the phagocytosis index values of macrophages in samples treated with transfer factors and those that were untreated ($p = 0.005$).

Conclusions: Transfer factors increased the phagocytic activity of macrophages in a Wistar rat model of peritonitis. This suggests that transfer factors could have a role as an immunomodulatory treatment for peritonitis.

1. Introduction

Peritonitis is an inflammation of the peritoneal membrane of the abdominal cavity and the organs within it [1–3]; it is a surgical emergency condition associated with a high level of infectious complications, such as sepsis, septic shock, and death [1,4–6].

The overall incidence of peritonitis is difficult to determine and varies according to the underlying abdominal disease process [1,7,8]. In Africa, between January 1993 and December 1999, it was reported that among 69 patients who underwent surgery, intestinal perforation was present in 35 (50.7%) and obstruction with or without intestinal gangrene was present in 9 (13%) [9,10].

Good management has played a role in reducing the incidence of peritonitis from 90% in 1900 to 23% in 2002 [5,7]. However, despite improvements in antimicrobial therapy, surgical techniques, and postoperative intensive care, peritonitis continues to cause high morbidity

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and mortality in pediatric patients [11,12].

There is a role for the immune system in improving the outcome of peritonitis cases in children [5,13]. In peritonitis, there is early activation of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1), and interferon gamma (IFN-γ), as well as recruitment of neutrophils followed by their replacement with macrophages [14–16]. The presence of bacterial infection on the peritoneal membrane also causes an immune response, including activation of macrophages that will phagocytose the bacteria [17]. Based on the immune-response mechanism, an alternative therapy could be the provision of additional immunomodulators [18]. Transfer factors is one immunomodulatory treatment that can increase the activity of natural killer (NK) cells to produce IFN-γ, which is thought to increase the phagocytic activity of macrophages [19]. This study analyzed the effect of transfer factors on the phagocytic activity of macrophages in the intraperitoneal fluid of a Wistar rat model of peritonitis.

2. Materials and methods

2.1. Study design

This experimental laboratory study with a post-test-only control group design was performed in a Wistar rat model at the Laboratory of Pharmacology and Microbiology, Padjadjaran University, Bandung, Indonesia. All animal procedures received approval from the local ethics commission. The work was carried out in line with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines [20].

2.2. Samples

The study population comprised 32 Wistar rats with peritonitis, which were randomly assigned to two groups: control and treatment. The inclusion criteria were age 8–10 weeks and bodyweight 200–350 g. Gender was not determined because peritonitis can occur in males and females. The Wistar rats developed peritonitis after being injected with *Escherichia coli* bacteria; the condition was confirmed by signs of piloerection, hyperpnea, and low motoric activity. The exclusion criteria were sickness during the adaptation period of 7 days (movement inactive) before being injected with E. coli and death during treatment.

2.3. Equipment and materials

- Materials for intraperitoneal injections, e.g., 70% alcohol and 2 ml E. *coli* at 2×10^8 CFU/ml.
- Materials for macrophage phagocytosis examination, e.g., chloroform, 70% alcohol, acetic acid 3%+crystal violet 1 mg/100 ml, Roswell Park Memorial Institute (RPMI)-1640 containing L-glutamine (l mM), 5% fetal bovine serum (FBS), 50 units of penicillin, and 50 μg/ml streptomycin, phosphate buffered saline (PBS), methanol, Giemsa stain, trypan blue, and sterile aquadest.
- Equipment, e.g., bent and straight scissors, tweezers, Bunsen burners, petri dishes, an enzyme-linked immunosorbent assay (ELISA) reader, a laminar flow unit, a Schwabach incubator, a colony counter, a microscope, syringes (3 cc and 10 cc), needles, centrifuge tubes (15 cc and 50 ml), sterile Pasteur pipettes, electronic scales, experimental animal cages, a temperature controlled centrifuge, thermometers, test-tubes, glassware, an autoclave, round slipcovers, and a high-performance liquid chromatography (HPLC) system (Shimadzu).

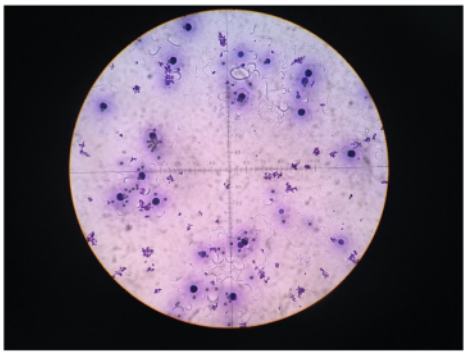

Fig. 1a. Macrophage phagocytic activity in latex beads from the control group under a light microscope (magnification 40 ×).

2.4. Procedures

1. Preparation

A total of 32 Wistar rats underwent an adaptation period of 7 days, during which they had access to food and water ad libitum. To avoid bias towards body weight, the animals were weighed before receiving treatment.

2. Injection procedure

The Wistar rats were placed in a supine position on a fixation pad, injected intraperitoneally with 2 ml E. *coli* bacteria at 2×10^8 CFU using a 3-cc syringe, and then observed for 1 day. Wistar rats with peritonitis showed signs of piloerection, hyperpnea, and low motor activity. The study animals were then divided into two groups (n = 16 for each) and housed individually [21].

3. Procedure for administration of transfer factors and antibiotics

The treatments given each group were as follows. For the K1 control group, the Wistar rats were injected intraperitoneally with *E. coli* and intramuscularly with ceftriaxone 6 mg. For the K2 treatment group, the Wistar rats were injected intraperitoneally with *E. coli* and intramuscularly with ceftriaxone 6 mg, and then given the transfer factor at a dose of 42 mg orally [22].

The transfer factor was given two times for 3 days using an oral pipette; the dose given was the maximum recommended for humans, it was given three times daily with two capsules at 385 mg multiplied by the conversion rate for 200 g bodyweight Wistar rats, which was 0.018. The antibiotic ceftriaxone at 6 mg was administered to both groups on days 9, 10, and 11. Transfer factor at a dose of 42 mg/day was given only to the treatment group [23–28].

4. Sampling procedure for intra-peritoneal macrophages
   - The Wistar rats were euthanized by dislocation of the cervical spine after the administration of anesthesia by chloroform, while lying in a supine position, and the entire surface of the stomach was doused with 70% alcohol.
   - Small incisions to the skin were made using scissors in the medial abdomen. Tweezers were used to peel back the skin towards both the head and the tail to reveal the peritoneum. The peritoneum was soaked with 70% alcohol to remove the hair.
   - RPMI medium (10 ml) containing 2% FBS was injected into the peritoneal cavity, which was then gently pressed for 2 min.
   - Peritoneal fluid was extracted from the peritoneal cavity by pressing on the internal organs using two fingers followed by aspiration using an injection syringe.
   - The aspirate was collected by centrifugation at 400×*g* and 4 °C for 10 min.
   - The supernatant was separated and washed twice with RPMI containing 2% FBS.

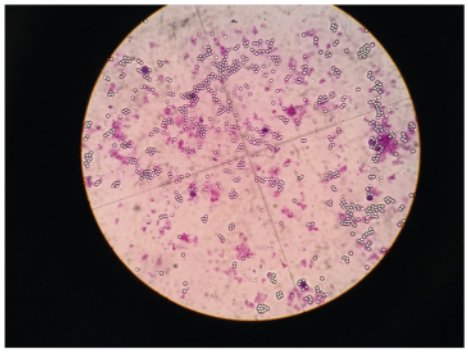

Fig. 1b. Macrophage phagocytic activity in latex beads from the treatment group under a light microscope (magnification 40 ×).

- The 2 ml of RPMI-1640 medium containing L-glutamine (1 nM), 5% FBS, 50 units of penicillin, and 50 μg of streptomycin were added, and the mixture was centrifuged at 400×g, 4 °C for 10 min.
- As necessary, the supernatant was dissolved in 3 acetic acid and FBS to lyse red blood cells, and then centrifuged at 400×g and 4 °C for 10 min.
- The sample was then washed in RPMI containing 2% FBS.
- This was followed by resuspension with complete medium.
- The number of cells was calculated using Neubauer counting chamber after staining with trypan blue to obtain a suspension with a density of 5×10^5 cells/ml.
- To examine phagocytic activity, latex beads were used at a concentration of 200 μl per well (24-microplate wells).

5. Examination of macrophage phagocytosis with latex beads

The macrophage phagocytic activity of latex particles was examined under light microscopy (Fig. 1A and B).

- The macrophage suspension was cultured in 24-well microplates with round cover slips, each of which contained 200 μl (5×10^5 cells), and then incubated in a 5% CO_2 incubator at 37 °C for 30 min.
- Complete medium (1 ml) was added to each well followed by incubation for 2 h.
- The cells were washed with RPMI twice, then complete medium was added (1 ml per well), followed by incubation for up to 24 h.
- Peritoneal macrophages were cultured the day before and washed twice with RPMI.
- Latex beads were suspended at a concentration of 2.5×10^7/ml.
- Latex suspension was added (200 μl per well) followed by incubation for 60 min at 37 °C in CO_2.
- Samples were washed three times with FBS to remove non-phagocytic particles.
- Samples were dried at room temperature, fixed with absolute methanol, and the cover slips were stained with Giemsa 20% for 30 min.
- Samples were washed in aquadest, removed from the culture well, and dried at room temperature.
- Samples were mounted on the object-glass.
- The number of latex particles phagocytosed by macrophages was counted under a light microscope.

2.5. Statistical analysis

Data analysis was performed using the SPSS version 20. Descriptive analysis was carried out by calculating the mean and median values as well as the data distribution (standard deviation [SD]), and then presented in tables and box plots. The normality test was the Shapiro–Wilk test. Differences in transfer factor administration and macrophage phagocytic activity levels were analyzed using the unpaired comparative *t*-test if the data were normally distributed or with the Mann-Whitney *U* test if the data were not normally distributed. The limit of the degree of significance was $p \leq 0.05$.

Table 1
Mean and median macrophage phagocytosis index values.

Group	N	Mean	±	SD	Median
Control (K1)	16	40,038	±	22,954	40,700
Treatment (K2)	16	59,931	±	13,067	59,600

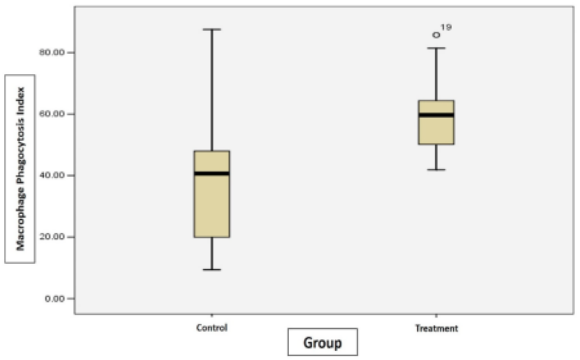

Fig. 2. Differences in macrophage phagocytosis index between the two groups.

Table 2
Normality test of control group and treatment group data on the macrophage phagocytosis index.

Group		Shapiro–Wilk test		
		Statistic	df	Sig.
Macrophage phagocytosis index	Control (K1)	.938	16	.325
	Treatment (K2)	.927	16	.219

3. Results

3.1. Macrophage phagocytosis index analysis

The phagocytic activity level of the macrophages increased after receiving the transfer factor in the treatment group (K2) compared to the control group, with mean values of 59.931 and 40.038, respectively (Table 1) (Fig. 2).

A normality test of the control group and treatment group data on the macrophage phagocytosis index with the Shapiro–Wilk test gave values of 0.325 and 0.219 respectively (Table 2). The values were >0.05 signifying that the data for the treatment group and control group were normally distributed. The results of the homogeneity test of variance were p-value > alpha or (0.089 > 0.05), indicating that the variance of the scores for the two data groups was homogeneous (Fig. 3A and B).

In this study, a significant difference was found between the phagocytosis index of macrophages from the samples were given transfer factor and those that were not, based on the independent sample *t*-test with a p-value of 0.005 ($p < 0.05$).

4. Discussion

Wistar rats infected with *E. coli* appeared to be less active. This could have been due to peritonitis caused by bacterial infection, resulting in the release of cytokines inducing cellular and humoral cascades that led

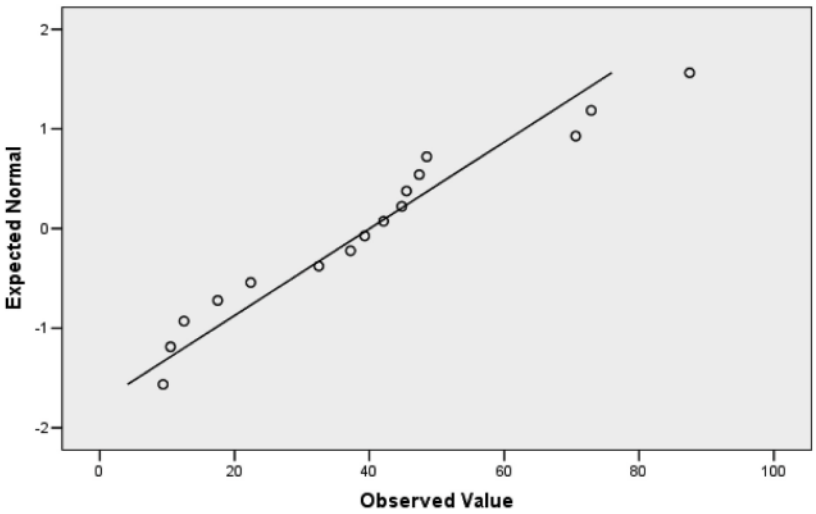

Fig. 3a. Q-Q plots of normality test results for the control group.

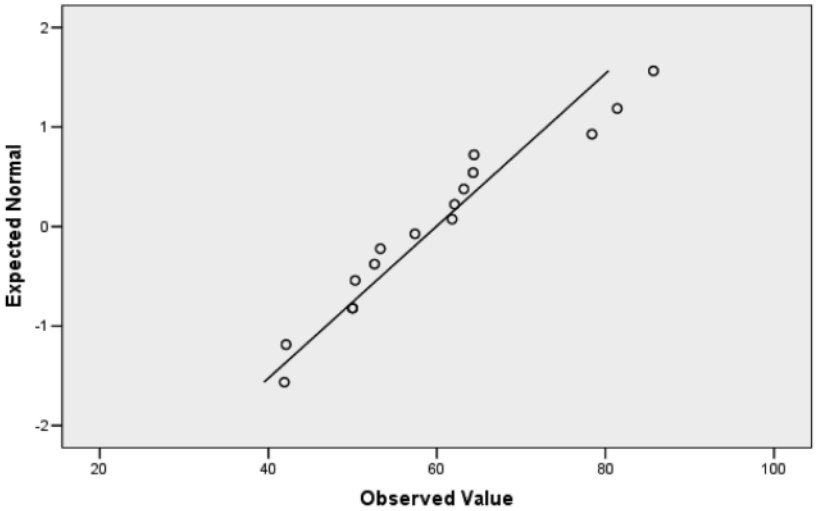

Fig. 3b. Q-Q plots of normality test results for the treatment group.

to cell damage, septic shock, and multiple organ dysfunction syndrome (MODS) [4,29].

In this study, Wistar rats that developed peritonitis after intraperitoneal injection of *E. coli* and were given a transfer factor showed a significant increase in macrophage phagocytic activity compared to the group that was not given a transfer factor. This could have occurred because the transfer factor plays a role in increasing the production and activity of NK cells, which in turn increases the production and activation of IFN-γ, as reported by El-Moiety et al. [30]. Furthermore, IFN-γ will increase the phagocytic activity of macrophages.

In the control group, there was also macrophage phagocytic activity because there was acute inflammation in the peritoneum and premature activation of pro-inflammatory cytokines such as TNF-α, IL-1, and IFN-γ. However, when compared to the treatment group, the phagocytic activity of macrophages in the control group was lower. Our study demonstrated that transfer factor administration clearly had a significant effect on increasing phagocytic activity in macrophages.

One function of the thymus is to act as the site of maturation of T cells derived from lymphocytes after they have migrated from their place of origin in peripheral lymphoid organs such as the spleen, lymph nodes, and Peyer's patches in the intestine [31]. If there is thymus deficiency in Wistar rats, there will be no maturation of T cells, so the transfer factor will be unable to increase the activity of macrophages; this is consistent with literature that has shown no clinical and laboratory changes in patients with thymus deficiency following transfer factor treatment [32].

Based on this study, further research is needed regarding the effect of transfer factor treatment on the phagocytic activity of macrophages in the management of peritonitis. Future studies should determine the leukocyte count, total lymphocytes, T lymphocytes, CD4 lymphocytes, and CD4/CD8 lymphocyte ratios in peritonitis through laboratory tests repeated one, six, and 12 months after the initiation of therapy. This could lead to clinical trials of transfer factors with known dosages that are tailored to meet not only the needs of specific patients but also to

selectively cope with the demands of peritonitis.

5. Conclusion

Transfer factors increased the phagocytic activity of macrophages in a Wistar rat model of peritonitis. Transfer factors have a role as an immunomodulatory treatment for peritonitis.

Ethical approval

The study was conducted after obtaining approval from the Ethics Commission of Padjajaran University, Bandung, Indonesia.

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This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contribution

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Registration of research studies

None.

Guarantor

Tommy R. Habar, Andi Asadul Islam, Mochammad Hatta, and Muhammad Nasrum Massi.

Consent

This manuscript does not involve human participants, human data, or human tissue.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Declaration of competing interest

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2021.102755.

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