# Arginine Supplementation Enhances Peritoneal Macrophage Phagocytic Activity in Rats with Gut-derived Sepsis

Yi-Yun Wang, MS[*], Huey-Fang Shang, PhD[+], Yu-Ni Lei, BS[*], Sung-Ling Yeh, PhD[*]

[*]Institute of Nutrition and Health Sciences, [+]Department of Microbiology and Immunology, Taipei Medical University, Taipei, Taiwan, ROC

Running title: effect of arginine on phagocytosis in sepsis

## Precis

Enteral Arginine supplementation before sepsis enhances macrophage phagocytic activity and reduces bacteria numbers in peritoneal lavage fluid. Arginine administered before and after sepsis have synergistic effect on enhancing phagocytosis and on bacterial clearance. However, intravenous Arginine administration after sepsis had no favorable effects on phagocytic activity or survival rates in gut-derived sepsis.

Corresponding author:
Sung-Ling Yeh, PhD
Institute of Nutrition and Health Science, Taipei Medical University
250 Wu Hsing Street, Taipei, Taiwan 110, Republic of China
Tel: 8862-27361661 ext. 6550*115
Fax: 8862-27373112
E-mail: sangling@tmu.edu.tw

ABSTRACT

***Background*:** Previous reports have shown that arginine (Arg) enhanced phagocytic activity of macrophage, and is required for macrophage-mediated toxicity toward tumor cells. Few studies have addressed the importance of Arg supplementation on macrophage and neutrophil function after infection and sepsis. This study examined the effect of Arg-supplemented diets before and Arg-enriched total parenteral nutrition (TPN) after sepsis or both on the phagocytic activity of peritoneal macrophages and blood polymorphonuclear cells in rats with gut-derived sepsis.

***Methods:*** Male Wistar rats were assigned to 4 groups. Groups 1 and 2 were fed a semipurified diet, while groups 3 and 4 had part of the casein replaced with 2% of total calories as Arg. After feeding the experimental diets for 10 days, sepsis was induced by cecal ligation and puncture (CLP); at the same time, an internal jugular vein was cannulated. All rats were maintained on TPN for 3 days. Groups 1 and 3 were infused with conventional TPN, while groups 2 and 4 were supplemented with Arg, replacing 10% of total amino acids in the TPN solution. Survival rates were recorded for 3 days after CLP, and all surviving rats were sacrificed 3 days after CLP to examine their immune responses.

***Results:*** Aerobic and anaerobic bacteria colony counts in peritoneal lavage fluid (PLF) were significantly reduced, and the phagocytic activity of peritoneal macrophages was enhanced in groups 3 and 4 but not in the other 2 groups. There were no significant differences in the phagocytic activities of blood polymorphonuclear cells and survival rates among the 4 groups.

***Conclusions:*** Enteral Arg supplementation before sepsis significantly enhanced peritoneal macrophage phagocytic activity and reduced total bacterial counts in PLF. Arg administered before and after CLP seemed to have synergistic effect on

enhancing phagocytic activity and on bacterial clearance. However, intravenous Arg administration after CLP had no favorable effects on phagocytic activity or survival rates in rats with gut-derived sepsis.

## INTRODUCTION

Arginine (Arg) is a semi-essential amino acid that has been shown to posses numerous useful physiologic properties.[1] Previous reports have revealed that supplemental dietary Arg reduces net protein catabolism and enhances the immune function in rats.[2-4] Also, Arg increases the mitotic response of peripheral blood lymphocytes to concanavalin A and phytohemmagglutinin both in normal humans[5] and in postoperative patients.[6] Although a recent meta-analysis of several studies focusing on immunonutrition indicated that Arg supplementation has no effect on infectious complications and may increase mortality in critically ill patients, immunonutrition with Arg is associated with a reduction in infectious complication rates and a shorter length of hospital stay without any adverse effect on mortality in elective surgical patients.[7] Arg is considered an essential amino acid for patients with catabolic diseases.[1,8]

Sepsis is a cause of death in patients with major surgery. Sepsis is initiated by bacteria and their related toxins. When bacterial toxins insult the body, profound alterations in both immune responses and organ functions may occur.[9] A study by Madden et al.[10] demonstrated that Arg given orally prior to injury or started intravenously after cecal ligation and puncture (CLP) improved the survival of rats. Also, Gianotti et al.[11] showed that Arg-supplemented diets improved survival in mice subjected to blood transfusions and peritonitis. The benefit of Arg appears to be mediated by improved bactericidal mechanisms via the Arg-nitric oxide (NO)

pathway.[11] NO is an intermediate metabolite generated during the biochemical transformation of Arg to citrulline. Stuehr and Marletta[12] noted that mouse peritoneal macrophages produce nitrite and nitrate when cultured with lipopolysaccharide. An *in vitro* study by Hibbs et al.[13] showed that the key role of Arg in macrophage- and lymphocyte-mediated toxicity toward infected cells is largely via the production and release of NO. A report by Tachibana et al.[14] showed that an Arg-enriched solution enhanced phagocytic activity of alveolar macrophages in tumor-bearing rats. However, few studies have addressed the importance of Arg supplementation on macrophage and neutrophil function after infection and sepsis.

Phagocytic cells are an essential arm of the host defense against microbes. Macrophages and neutrophils are involved in the early, nonspecific host-defense responses and play an important role in the pathophysiology and/or protection against sepsis.[15,16] To our knowledge, the effects of Arg on phagocytic activity in gut-origin sepsis have not been previously studied. Therefore, our aim was to investigate the effect of Arg supplementation before and after sepsis and both before and after on the ability of the host defense to kill translocated enteric bacteria in the peritoneal cavity and organisms that invade the systemic circulation. In this study, we used CLP as a sepsis model, because CLP is more clinically relevant and is considered to be a simple and reproducible model of gut-derived sepsis in rats.[17]

## CLINICAL RELEVANCY STATEMENT

In this study, we found that enteral Arg supplementation before sepsis significantly enhances peritoneal macrophage phagocytic activity and reduces total bacteria numbers in peritoneal lavage fluid. Arg administered both before and after CLP seemed to have a synergistic effect on enhancing phagocytic activity and on bacterial

clearance. However, intravenous Arg administration after CLP had no favorable effects on phagocytic activity or survival rates in rats with gut-derived sepsis. The phagocytic activity of blood polymorphonuclear cells did not differ among the Arg-supplemented and control groups. This result may indicate that Arg augments phagocytic activity at the location of bacterial invasion. The effect of Arg on phagocytic cells in systemic circulation was not obvious.

## MATERIALS AND METHODS

### *Animals*

Male Wistar rats weighing 200-230 g were used in this study. All rats were housed in temperature- and humidity-controlled rooms and were allowed free access to a standard rat chow for 3 days prior to the experiment. The care of the animals used in this study followed standard experimental animal care procedures.

### *Study protocol*

All rats were assigned to 4 groups. Groups 1 and 2 were fed a semipurified diet. Rats in groups 3 and 4 were fed an identical diet except that part of the casein was replaced by Arg, which provided 2% of the total energy intake (Table 1). After feeding the experimental diets for 10 days, sepsis was induced by CLP according to the method of Wichterman et al.[17] Briefly, rats were anesthetized with intraperitoneal pentobarbital (50 mg/kg), and the abdomen was opened through a midline incision. The cecum was isolated, and a 3-0 silk ligature was placed around it, ligating the cecum just below the ileocecal valve. The cecum was then punctured twice with an 18-gauge needle and was replaced back into the abdomen. The abdominal wound was closed in 2 layers. Immediately after CLP, the right internal

jugular vein was cannulated with a Silastic catheter (Dow Corning, Midland, MI, USA). The catheter was tunneled subcutaneously to the back of the neck, and exited through a coiled spring, which was attached to a swivel allowing free mobility of animals inside individual metabolic cages. Two milliliters per hour of total parenteral nutrition (TPN) was administered on the first day. Full strength TPN was given thereafter. The infusion speed was controlled by a Terufusion pump (Model STC-503, Terumo, Tokyo, Japan). The TPN solution without fat was prepared in a laminar flow hood. Sterilized fat emulsions were added to the TPN solution daily just before use. The TPN solution was infused for the entire day at room temperature. All rats were maintained with TPN for 3 days. Groups 1 and 3 were infused with conventional TPN. Groups 2 and 4 were supplemented with Arg, replacing 10% of total amino acids, which also provided 2% of the total energy of the TPN solution. TPN provided 280 kcal/kg body weight, and the kcal/nitrogen ratio was 119:1. The TPN solutions were isonitrogenous and identical in nutrient composition except for the difference in amino acid content (Table 2). There were 4 groups: group 1, without Arg supplementation before and after CLP (-/-); group 2, Arg-supplemented TPN after CLP (-/+); group 3, Arg-supplemented diet before CLP (+/-); group 4, Arg supplemention before and after CLP (+/+).

*Measurements and analytical procedures*

Survival rates were noted daily after CLP, and were recorded for 3 days. TPN was continued until the time of sacrifice at day 3 after CLP, and surviving rats were weighed and anesthetized. A middle abdominal incision was made, and 10 ml of phosphate-buffered saline (PBS) was intraperitoneally injected to elute the peritoneal cells. After harvesting the peritoneal lavage fluid (PLF), the rats were sacrificed by drawing arterial blood from the aorta of the abdomen. Fresh PLF was used for

bacteriological analysis and macrophage phagocytic activity, and the remaining samples were stored at –80 ℃ until assay for NO and tumor necrosis factor (TNF)-α. Blood samples were collected in tubes containing heparin. Whole blood was used for analyzing bacterial counts, and neutrophil phagocytosis was determined by flow cytometry. Plasma was centrifuged from the remaining blood, and was stored at -80 ℃ for analysis of NO and amino acid concentrations.

*Technique for quantitative bacteriologic culture*

A total aerobic bacterial count was made by spreading 50 μl of whole blood or ten-fold serially diluted PLF on TSA blood agar plates （BBL® media, Becton Dickinson, Sparks, NJ）and incubating at 37 ℃ overnight. A total anaerobic bacteria count was made by spreading 50 μl of the above samples on CDC blood agar plates (BBL® media）and incubating under anaerobic conditions (GasPak System, Becton Dickinson Microbiology Systems) at 37 ℃ for 2 days. The total number of colonies formed on each plate was counted. The results were expressed as colony forming units (CFU) per milliliter of blood or PLF.

*Phagocytosis assay of blood polymorphonuclear neutrophils (PMNs)*:[18,19]

A flow cytometric phagocytosis test was used to evaluate the phagocytic activity of blood PMNs. One hundred microliters of heparinized whole blood was aliquoted on the bottom of a 12 x 75-mm Falcon polystyrene tube (Becton Dickinson) and placed in an ice water bath. Twenty microliters of precooled opsonized FITC-labeled *E. coli* (Molecular Probes, Eugene, Oregon, USA.) was added to each tube. Control tubes remained on ice, and assay samples were incubated for precisely 10 min at 37 ℃ in a shaking water bath. After incubation, samples were immediately placed in ice water, and 100 μL of a precooled trypan blue (Sigma, St. Louis, MO, USA) solution (0.25 mg/ml in citrate salt buffer; pH 4.4) was added to

quench the fluorescence of the bacteria merely adhering to the surface of the phagocytizing cells. Cells were washed twice in Hanks buffered saline (HBSS), and erythrocytes were lysed by the addition of FACS lysing solution (Becton Dickinson). After an additional wash in HBSS, 100 μL of propidium iodide (PI) solution (1μg/mL in HBSS) was added to stain the nuclear DNA 10 min before the flow cytometric analysis. Flow cytometry was performed on a FACSCalibur™ flow cytometer (Becton Dickinson) equipped with a 488-nm argon laser. A live gate was set on the red (PI) fluorescence histogram during acquisition to include only those cells with a DNA content at least equal to human diploid cells. The number of cells with phagocytic activity did not exceed 6% at 0 °C.

*Phagocytosis assay of peritoneal macrophages*

A Vybrant ™ Phagocytosis Assay kit (Molecular Probes) was used to evaluate the phagocytic activity of peritoneal macrophages. After washing the peritoneal macrophages 3 times with HBSS, the cell concentration was counted, and the cell number was adjusted to $10^6$ cells/ml with RPMI-1640 supplemented with 5% fetal bovine serum and an adequate amount of antibiotics. After distributing 100 μl of diluted solutions into each well on a 96-well microplates it was transferred to a 37 ℃ $CO_2$ incubator for 1 h to allow the cells to adhere to the microplate surface. The RPMI solution was removed from all microplate wells by vacuum aspiration, and then 100 μl of the prepared FITC-labeled *E. coli* was added to each well for 2 h. Labeled bacteria was removed by vacuum aspiration, and 100 μl of trypan blue suspension was added to all wells within 1 min. The excess trypan blue was immediately aspirated, and the experimental and control wells (without peritoneal macrophages) were read in the fluorescence plate reader using ~480 nm for excitation and ~520 nm for emission.

*Plasma amino acid analysis*

Plasma amino acids were analyzed by standard ninhydrin technology (Beckman Instruments, model 6300, Palo Alto, CA), after deproteinization of the plasma with 5% salicylic acid.[20]

*Measurement of TNF-α concentration*

Concentrations of TNF-α in PLF were determined with a commercially available enzyme-linked immunosorbent assay in microtiter plates. Antibodies specific for rat TNF-α were coated onto the wells of the microtiter strips provided (Amersham Pharmacia Biotech, Buckinghamshire, UK).

*Determination of NO*

NO is highly unstable in solution and cannot be readily assayed. However, NO is converted to stable nitrite and nitrate ions in aqueous solution. After conversion of nitrate to nitrite using nitrate reductase, nitrite concentrations were measured using the Griess reagent. The concentrations of $NO_2^-/NO_3^-$ in plasma and PLF were determined with a commercial kit (Assay Designs, Ann Arbor, MI, USA). Procedures followed the manufacturer's instructions.

*Statistical Analysis*

Data are expressed as the mean ± SD. Differences among groups were analyzed by ANOVA using Duncan's test. A $p$ value of $< 0.05$ was considered statistically significant.

RESULTS

There were no differences in initial body weights and body weights after feeding the experimental diets for 10 days, and after TPN administration for 3 days

among the 4 groups (data not shown). The Arg-supplemented groups (group 2, 168.6 ± 59.3; group 3, 159.6 ± 24.1; group 4, 178.4 ± 77.2 nmol/mL) had significantly higher plasma Arg levels than did group 1 (71.6 ± 31.5 nmol/mL). There were no differences in plasma citrulline concentrations among the 4 groups (data not shown).

The numbers of total aerobic or anaerobic bacteria in PLF of groups 3 (+/-) and 4 (+/+) were significantly less than those of groups 1 (-/-) and 2 (-/+). The total bacteria numbers in the whole blood did not differ among the 4 groups (Fig. 1). No significant difference in the cell numbers of peritoneal macrophages was observed among the 4 groups (data not shown). Phagocytic activities were significantly higher in groups 3 (+/-) and 4 (+/+) than in groups 1 (-/-) and 2 (-/+) (Fig. 2). There were no significant differences in the phagocytic activities of blood PMNs among the 4 groups (Fig 3).

Concentrations of $NO_2^-/NO_3^-$ in plasma and PLF did not differ among the 4 groups 3 days after CLP (Fig. 4). There were no significant differences in PLF concentrations of TNF-α among the 4 groups (Fig. 5). No significant differences in survival rates were observed among the all groups (Fig. 6).

## DISCUSSION

In this study, 2% of total energy was supplied by Arg. This amount of Arg was found to reduce mortality in rodents in gut-derived sepsis.[11] We provided oral Arg supplementation for 10 days before sepsis induction. This model mimics the septic complications in patients with abdominal surgery, in whom preventive use of an Arg-supplemented enteral diet may be recommended. TPN was administered after CLP, because sepsis has been shown to adversely affect the barrier and metabolic functions of the small intestine, as well as to reduce mesenteric blood flow and cause

histologic damage.[21,22] A study by Gardiner et al.[23] showed that sepsis induced by CLP resulted in impaired intestinal amino acid uptake, and the parenteral rather than the enteral route of Arg therapy may have benefits for survival from septic insult.

The results of this study showed that the phagocytic activity in peritoneal macrophages was much higher in groups with Arg supplementation before CLP (groups 3 and 4), and aerobic and anaerobic bacteria in PLF were significantly reduced in groups 3 and/or group 4. CLP causes peritoneal contamination with mixed bacteria flora, mostly anaerobic bacteria originating from enteric organisms. These findings indicate that Arg supplementation enhanced the activity of peritoneal macrophages, and may consequently modulate bacterial clearance at the site of injury. We did not analyze enteric organism quantitatively or qualitatively in this study, whether Arg supplementation before CLP resulted in the change of intestinal flora, thus play roles in decreasing bacteria counts in PLF needs to be clarified.

Hibbs et al.[24] demonstrated that L-Arg is required *in vitro* for the cytotoxic effector mechanisms of macrophages, and that the production of NO via the conversion of Arg to citrulline is controlled by the Arg deaminase pathway. In this study, $NO_2^-/NO_3^-$ concentrations in plasma and PLF did not differ among the 4 groups. Plasma citrullin levels not differring among the 4 groups suggests that NO synthetase activities were not stimulated and NO was not induced after Arg supplementation. It is possible that NO synthesis in response to metabolic stress such as with sepsis in this study was already at a peak.[25] This result is similar to that of a study by Cui et al.[26], in which they also found no difference in the plasma NO product between an Arg-supplemented group and a control group after burn injury. Since we did not analyze NO levels within macrophages, whether NO derived from Arg in macrophages is responsible for enhancing phagocytic activity requires further

investigation.

TNF-α is a macrophage-derived peptide that has antitumor action and modulates immune and inflammatory reactions.[27] Previous reports showed that high plasma TNF-α levels were associated with increased severity of inflammatory diseases.[27,28] In this study, there were no differences in PLF TNF-α concentrations among the groups. This finding may indicate that the severity of the inflammatory reaction was similar among the groups, and that Arg supplementation did not modulate the TNF-α production of peritoneal macrophages. Because interleukin-1 and TNF-α can both activate macrophage activity,[28] whether IL-1 plays role in enhancing the phagocytic activity of peritoneal macrophages is not clear.

Results of phagocytic activity of peritoneal macrophages, and of bacterial counts in PLF showed that there were no differences between groups 3 (+/-) and 4 (+/+), whereas those of group 2 (-/+) were significantly lower than those of group 3 and were no different from those of group 1 (-/-). This finding may indicate that Arg supplementation before sepsis had beneficial effects on enhancing phagocytic activity. Arg administered both before and after CLP seemed to have a synergistic effect on phagocytic activity and bacterial killing. No favorable effects were noted when Arg was supplemented after CLP. This result is inconsistent with that of a report by Madden et al.[10] Their report showed that intravenously administered Arg after CLP significantly increased animal survival over those without Arg. The dosage per day used in this study was comparable to that of Madden et al. However, in that study 100 mg of Arg hydrochloride was infused intravenously 3 times a day, and the Arg dosage injected at the given times was high. In this study, we provided Arg evenly in 24 h via TPN, and the plasma Arg concentration was relatively constant. Whether a high-dose injection of Arg in a very limited time may activate the immune response

and improve the survival after CLP requires further investigation.

PMNs are potent inflammatory cells, and both the total number and percentage of circulating PMNs can be induced by acute infection and endotoxin.[15] PMNs efficiently engulf a wide variety of microbes. In this study, the phagocytic activity of blood PMNs cells did not differ among the 4 groups. Also, there were no differences in blood total aerobic and anaerobic bacteria numbers among the groups. Since the peritoneal cavity is the primary site of injury, it is possible that Arg augments phagocytic activity at the location of bacterial invasion. The effect of Arg on phagocytic cells in the systemic circulation was not obvious.

In summary, this study shows that enteral Arg supplementation before sepsis significantly enhances peritoneal macrophage phagocytic activity; the total bacteria number in PLF was also reduced. Arg administered before and after CLP seemed to have a synergistic effect on phagocytic activity and bacterial clearance at the site of injury. However, intravenous Arg administration after CLP had no favorable effects on phagocytic activity or on survival in rats with gut-derived sepsis.

## ACKNOWLEDGEMENTS

This study was supported by research grant NSC 91-2320-B-038-017 from the National Science Council, ROC.

REFERENCES

1. Evoy D, Lieberman MD, FaheyIII TJ, et al: Immunonutrition: the role of arginine. Nutrition 4:611-617, 1998
2. Barbul A, Wasserkrug HL, Yoshimura NN, et al: High arginine levels in intravenous hyperalimentation abrogate post-traumatic immune suppression. J Surg Res 36:620-624, 1984
3. Sietger E, Rettura G, Barbul A, et al: Arginine an essential amino acid for injured rats. Surgery 84:224-230, 1978
4. Sitren HS, Fisher H: Nitrogen retention in rats fed on diets enriched with arginine and glycine. I. Improved N retention after trauma. Br J Nutr 37:195-208, 1977
5. Barbul A, Sisto D, Wasserkrug H, et al: Arginine stimulates lymphocyte immune response in healthy human beings. Surgery 90:244-251, 1981
6. Daly JM, Reynolds J, Thom A, et al: Immune and metabolic effects of arginine in surgical patients. Ann Surg 208:512-523, 1988
7. Heyland DK, Novak F, Drover JW, et al: Should immunonutrition become routine in critically ill patients? A systematic review of the evidence. JAMA 286:944-953, 2001
8. De-Souza DA, Greene LJ: Pharmacological nutrition after burn injury. J Nutr 128:797-803, 1998
9. Zellweger R, Ayala A, DeMaso CM, et al: Trauma-hemorrhage causes prolonged depression in cellular immunity. Shock 4:149-153, 1995
10. Madden HP, Breslin RJ, Wasserkurg HL, et al: Stimulation of T cell immunity by arginine enhances survival in peritonitis. J Surg Res 44:658-663, 1988
11. Gianotti L, Alexander JW, Pyles T, et al: Arginine-supplemented diets improve survival in gut-derived sepsis and bacterial clearance. Ann Surg 217:644-653,

1993

12. Stuehr DJ, Marletta MA: Mammalian nitrate biosynthesis: mouse macrophages produce nitrite and nitrate in response to *Escherichia coli* lipopolysaccharide. Proc Natl Acad Sci USA 82:7738-7743, 1985
13. Hibbs JB, Vavrin Z, Taintor RR: L-Arginine is required for expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. J Immunol 138:550-555, 1987
14. Tachibana K, Mukai K, Hiraoka I, et al: Evaluation of the effect of arginine-enriched amino acid solution on tumor growth. J Parenter Enter Nutr 9:428-434, 1985
15. Ringer TV, Zimmermann JJ: Inflammatory host responses in sepsis. Crit Care Clin 8:163-189, 1992
16. Sawyer DW, Donowitz DR, Mandell GL: Polymorphonuclear neutrophils: an effective antimicrobial force. Rev Infect Dis 11:1532-1544, 1989
17. Wichterman KA, Baue AE, Chaudry IH: Sepsis and septic shock-a review of laboratory models and a proposal. J Surg Res 29:189-201, 1980
18. Böhmer RH, Trinkle LS, Staneck JL: Dose effects of LPS on neutrophils in a whole blood flow cytometric assay of phagocytosis and oxidative burst. Cytometry 13: 525-531, 1992
19. Schiffrin EJ, Rochat F, Aeschlimann JM, et al: Immunomodulation of human blood cells following ingestion of lactic acid bacteria. J Dairy Sci 78:491-497, 1995
20. Smith RJ, Panico K: Automated analysis of o-phthalaldehyde derivatives of amino acids in physiological fluids of reverse phase high performance liquid chromatography. J Liq Chromatogr 8:1783-1795, 1985

21. Navaratnam RLN, Morris SE, Traber DL, et al: Endotoxin (LPS) increases mesenteric vascular resistance (MVR) and bacterial translocation (BT). J Trauma 30:1104-1113, 1990
22. Gardiner K, Barbul A: Intestinal amino acid absorption during sepsis. J Parenter Enter Nutr 17:277-283, 1993
23. Gardiner KR, Gardiner RE, Barbul A: Reduced intestinal absorption of arginine during sepsis. Crit Care Med 23:1227-1232, 1995
24. Hibbs JB, Taintor RR, Vavrin Z: Macrophage cytotoxicity: role for L-arginine deaminase and imino nitrogen oxidation to nitrate. Science 235:473-476, 1987
25. Lindblom L, Cassuto J, Yregard L, et al: Role of nitric oxide in the control of burn perfusion. Burns 26:19-23, 2000
26. Cui XL, Iwasa M, Iwasa Y, et al: Arginine-supplemented diet decreases expression of inflammatory cytokines and improves survival in burned rats. J Parenter Enter Nutr 24:89-96, 2000
27. Beutler B, Cerami A: Tumor necrosis, cachexia, shock and inflammation: a common mediator. Annu Rev Biochem 57:505-518, 1988
28. DiPiro JT: Cytokine networks with infection: mycobacterial infections, leishmaniasis, human immunodeficiency virus infection, and sepsis. Pharmacotherapy 17:205-223, 1997

FIGURE LEGENDS

Fig. 1. Total counts of aerobic and anaerobic bacteria in whole blood and peritoneal lavage fluid (PLF) in the 4 groups 3 days after CLP. Aerobic bacteria were isolated on TSA blood agar plates and cultured in a 37℃ incubator for 24 h. Anaerobic bacteria were isolated on CDC blood agar plates and cultured in Gas-Pak jar at 37℃ for 48 h. The numbers of total aerobic or anaerobic bacteria in PLF of groups 3 and 4 were significantly less than those of groups 1 and 2 ($p < 0.05$). Total numbers of bacteria in whole blood did not differ among the 4 groups ($p > 0.05$).(group 1, n = 12; group 2, n = 13; group 3, n =12; group 4, n = 11).

Fig. 2. Phagocytic activity of peritoneal macrophages. After subtraction the background of the control wells, the phagocytic activities of groups 3 (+/-) and 4 (+/+) were significant higher than those of groups 1 (-/-) and 2 (-/+) ($p < 0.05$). (group 1, n = 8; group 2, n = 11; group 3, n =9; group 4, n = 9).

Fig. 3. Tumor necrosis factor (TNF)-α concentrations in peritoneal lavage fluid of the 4 groups 3 days after CLP. There were no significant differences among the 4 groups ($p > 0.05$). (group 1, n = 8; group 2, n = 11; group 3, n =9; group 4, n = 9).

Fig. 4. Nitric oxide (NO) concentrations in plasma and peritoneal lavage fluid (PLF) of the 4 groups 3 days after CLP. There were no significant differences among the 4 groups ($p > 0.05$). (group 1, n = 7; group 2, n = 7; group 3, n = 9; group 4, n = 9).

Fig. 5. Phagocytic activity of peripheral blood neutrophils by flow cytometry. No significant differences were observed among the 4 groups ($p > 0.05$). (group 1, n = 8; group 2, n = 10; group 3, n =10; group 4, n = 9).

Fig. 6. Survival rates for 3 days of animals with Arg supplementation before and after and both before and after CLP. Survival rates did not differ among the 4 groups ($p > 0.05$). (group 1, n = 10; group 2, n = 10; group 3, n =10; group 4, n = 10).

Table 1. Composition of the semipurified diet (g/kg)

| Ingredients | Arg-supplemented | Without Arg |
|---|---|---|
| Casein | 180 | 220 |
| Arg | 20 | -- |
| Total nitrogen | 35.2 | 35.2 |
| Corn starch | 677 | 657 |
| Soybean oil | 44 | 44 |
| Vitamin [a] | 10 | 10 |
| Salt mixture [b] | 35 | 35 |
| Methyl-cellulose | 30 | 30 |
| Choline chloride | 1 | 1 |
| DL-methionine | 3 | 3 |

**[a]** The salt mixture contains the following (mg/g): calcium phosphate diabasic 500, sodium chloride 74, potassium sulphate 52, potassium citrate monohydrate 220, magnesium oxide 24, manganese carbonate 3.5, ferric citrate 6, zinc carbonate 1.6, cupric carbonate 0.3, potassium iodate 0.01, sodium selenite 0.01, and chromium potassium sulphate 0.55.

[b] The vitamin mix contains the following (mg/g): thiamin hydrochloride 0.6, riboflavin 0.6, pyridoxine hydrochloride 0.7, nicotinic acid 3, calcium pantothenate 1.6, D-biotin 0.02, cyanocobalamin 0.001, retinyl palmitate 1.6, DL-α-tocopherol acetate 20, cholecalciferol 0.25, and menaquinone 0.005.

Table 2. Composition of the TPN solution (mL/L)

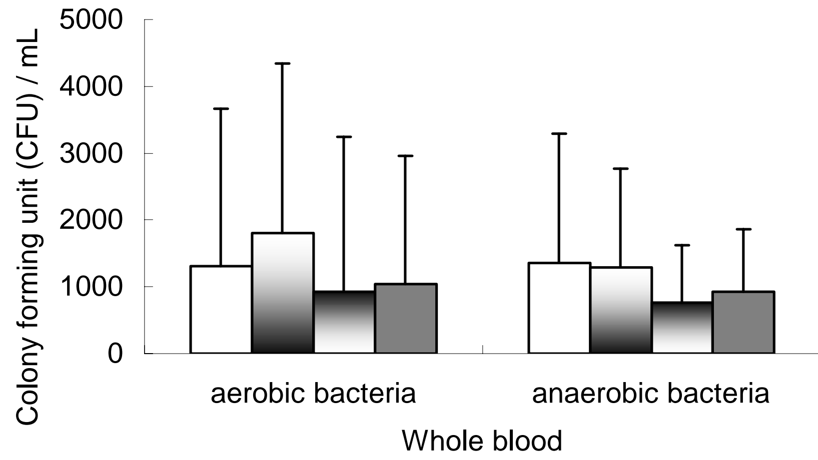
| | Arg-supplemented | Without Arg |
|---|---|---|
| Glucose 50% | 412 | 400 |
| Fat emulsion 20% | 50 | 50 |
| Moriamin-SN 10%* | 450 | 556 ml |
| Arg | 5g | -- |
| Infuvita** | 8 | 8 |
| NaCl 3% | 35 | 35 |
| KCl 7% | 10 | 10 |
| $K_3PO_4$ 8.7% | 10 | 10 |
| Ca-gluconate | 10 | 10 |
| $MgSO_4$ | 4 | 4 |
| Zn $SO_4$ | 2 | 2 |
| Choline chloride (g) | 1 | 1 |

* From Chinese Pharmaceuticals, Taipei, Taiwan

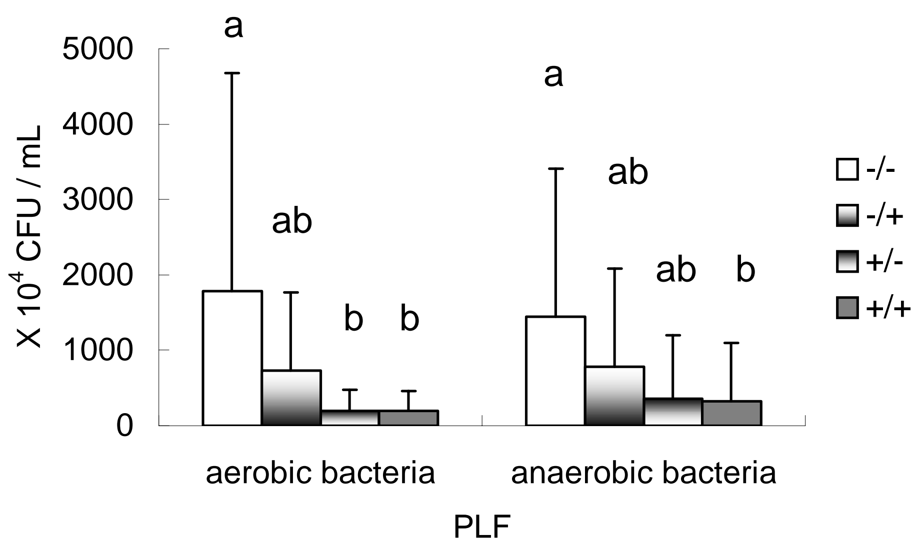
Per deciliter contains: Leu 1250 mg, Ile 560 mg, Lys acetate 1240 mg, Met 350 mg, Phe 935 mg, Thr 650 mg, Trp 130 mg, Val 450 mg, Ala 620 mg, Arg 790 mg, Asp 380 mg, Cys 100 mg, Glu 650 mg, His 600 mg, Pro 330 mg, Ser 220 mg, Tyr 35 mg, Aminoacetic acid (Gly) 1570 mg.

**From Yu-Liang Pharmaceuticals, Taoyuan, Taiwan

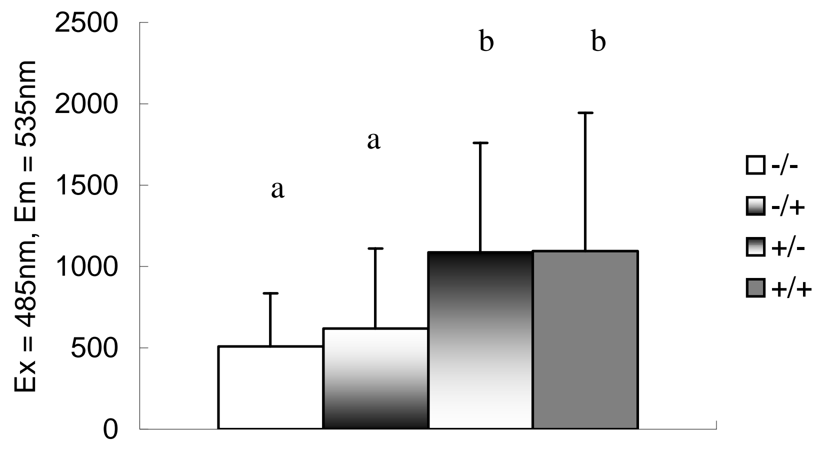
Per milliliter contains: ascorbic acid 20 mg, vitamin A 660 IU, ergocalciferol 40 IU, thiamine HCl 0.6 mg, riboflavin 0.72 mg, niacinamide 8 mg, pyridoxine HCl 0.8 mg, D-panthenol 3 mg, dl-$\alpha$-tocopheryl acetate 2 mg, biotin 12 ug, folic acid 80 ug, cyanocobalamin 1 ug.

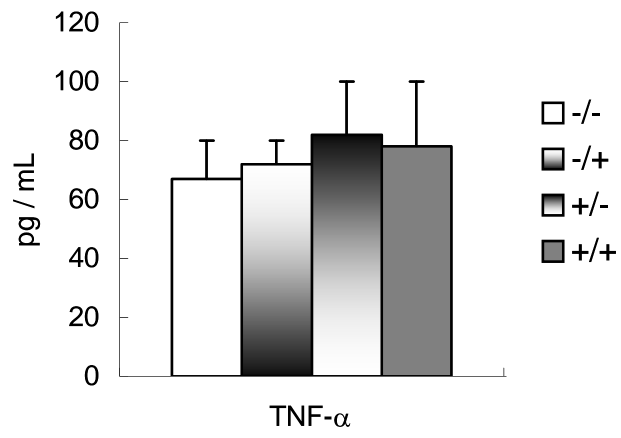


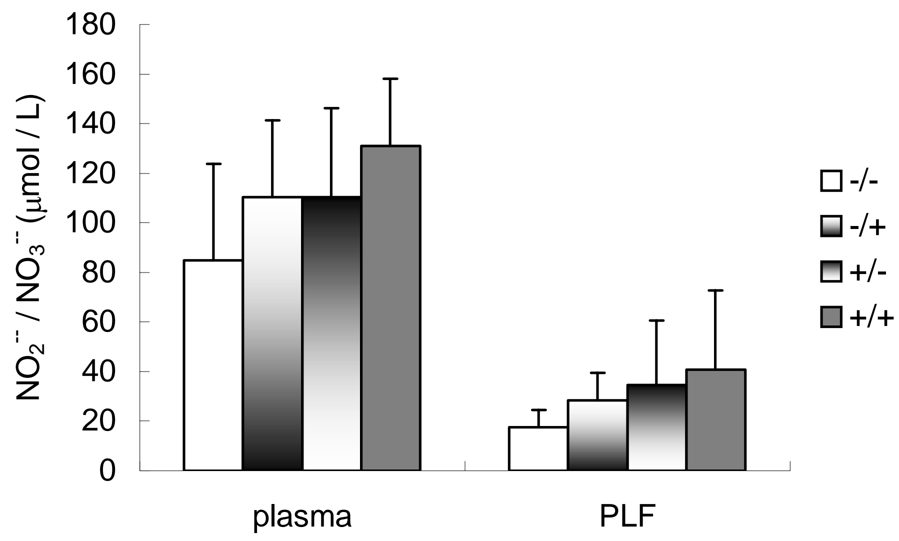
Colony forming unit (CFU) / mL
5000
4000
3000
2000
1000
0
aerobic bacteria
anaerobic bacteria
Whole blood

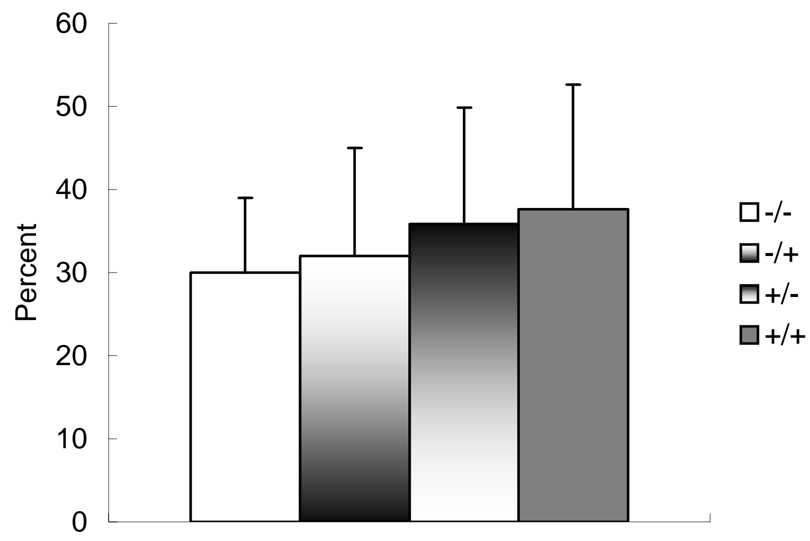
X 10^4 CFU / mL
5000
4000
3000
2000
1000
0
a
ab
b
b
a
ab
ab
b
aerobic bacteria
anaerobic bacteria
PLF
-/-
-/+
+/-
+/+

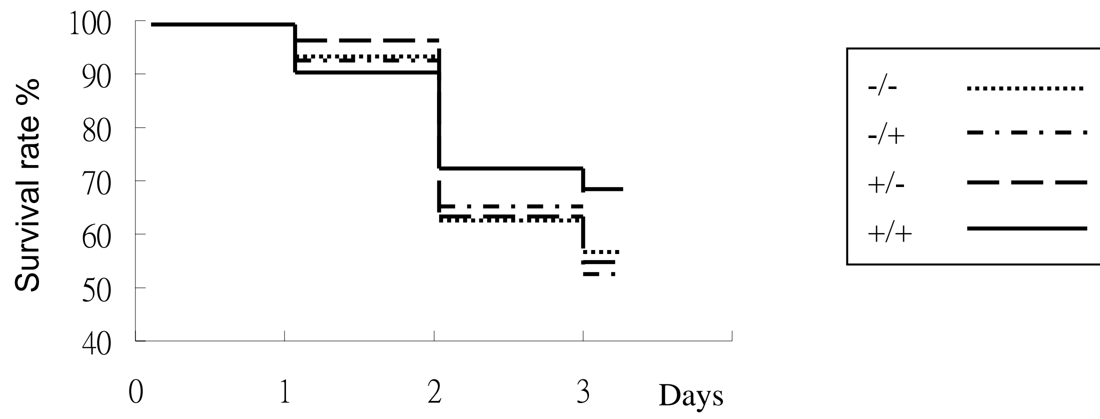

2500
2000
1500
1000
500
0
Ex = 485nm, Em = 535nm
a
a
b
b
-/-
-/+
+/-
+/+


120
100
80
60
40
20
0
pg / mL
TNF-α
-/-
-/+
+/-
+/+



180
160
140
120
100
80
60
40
20
0
NO2⁻ / NO3⁻ (μmol / L)
plasma
PLF
-/-
-/+
+/-
+/+


60
50
40
30
20
10
0
Percent
-/-
-/+
+/-
+/+


100
90
80
70
60
50
40
Survival rate %
0
1
2
3
Days
-/-
-/+
+/-
+/+