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Effects of the Antioxidant-Enriched Concentrated Liquid Diet ANOM on Oxidative Stress and Multiple Organ Injury in Patients with Septic Shock: A Pilot Study

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Abstract

Purpose: In patients with septic shock, excessive production of reactive oxygen species (ROS) by activated neutrophils injures the vascular endothelium. The body responds by recruiting systemic antioxidants, among which vitamin C most rapidly responds. Excessive and prolonged ROS production quickly depletes vitamin C, which is not synthesized by the human body. To improve the prognosis of patients with septic shock, we evaluated the ability of an antioxidant-enriched liquid diet to alleviate oxidative stress and multiple organ failure.

Methods: We evaluated 15 patients with septic shock complicated by multiple organ failure who underwent treatment with ANOM® (antioxidant-enriched concentrated liquid diet, Otsuka Pharmaceutical Co, Tokushima, Japan). Vitamin C concentration was determined by measuring serum levels of vitamin C radicals (VCR) with electron spin resonance (ESR) spectroscopy.

Results: Serum VCR concentration was significantly increased from the baseline level of 0.155±0.026 after 1 day of ANOM infusion, reaching the normal range at day 3, and 0.642±0.059 at day 7. Serum 8-hydroxydeoxyguanosine (8-OHdG) concentration, a measure of ROS-induced oxidative injury, was 0.495±0.061 ng/mL at day 7, which was significantly lower than the baseline level of 0.896±0.065 ng/mL. The Sepsis-related Organ Failure Assessment (SOFA) score, a measure of multiple organ failure, decreased significantly from 10.9±1.9 at baseline to 6.2±1.7 at day 7.

Conclusions: These findings show that ANOM rapidly restores vitamin C levels, suggesting that it protects against excessive oxidative stress and alleviates multiple organ failure in patients with septic shock.

Keywords: Oxidative stress; Vascular endothelial injury; Vitamin C; ANOM; Serum 8-OHdG

Introduction

In patients with septic shock, bacterial invasion triggers the production of inflammatory cytokines by monocytes and macrophages, resulting in systemic activation of vascular endothelial cells and neutrophils. Activated neutrophils firmly adhere to the vascular endothelium and release large amounts of reactive oxygen species (ROS) such as superoxide [1]. Inflammatory cytokines upregulate inducible nitric oxide synthase in cells throughout the body to generate reactive nitrogen species (RNS) such as nitric oxide [2]. The body responds by rapidly recruiting stored antioxidants to scavenge ROS/RNS; however, excessive ROS/RNS production overwhelms the antioxidant system because the antioxidants that cannot be synthesized by the body (i.e., antioxidant vitamins) are quickly depleted, resulting in oxidative stress [3,4].

If not controlled, the high levels of ROS/RNS cause oxidation of normal tissues, lipid peroxidation, protein degradation, and oxidative DNA damage. Thus, oxidative stress damages cell membranes rich in unsaturated fatty acids, impairs enzyme/receptor function, alters gene expression, and induces apoptosis, leading to systemic injury to the vascular endothelium [5]. This cellular damage causes microcirculatory disorder, ultimately leading to multiple organ failure [5]. More severe oxidative stress is associated with worse prognosis and higher mortality [7-9]. These findings emphasize the importance of protecting the vascular endothelium, not only by treating the underlying disease responsible for excessive ROS production, but also through antioxidant supplementation at its onset. However, moderate levels of ROS/RNS are critical to protect against pathogen invasion, and excessive antioxidant supplementation may account for the failure of prophylactic administration of antioxidants to improve rates of mortality from various diseases [10,11]. Thus, successful antioxidant replacement therapy requires a balance between ROS/RNS production and the antioxidant system.

The antioxidant-enriched concentrated liquid diet ANOM® has been used clinically in Japan since 2006. In this study, we assessed the ability of ANOM to ameliorate oxidative stress and organ failure in patients with septic shock by measuring vitamin C radical (VCR) levels in real time to assess vitamin C status [15,16], measuring serum 8-hydroxydeoxyguanosine (8-OHdG), a sensitive biomarker of ROS/RNS-induced oxidative injury, and calculating the Sepsis-related Organ Failure Assessment (SOFA) score, an index of multiple organ failure.

Materials and Methods

Patient treatment

The effects of the antioxidant-enriched ANOM diet on oxidative stress

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and progression of multiple organ failure were investigated in patients with septic shock admitted to the intensive care unit (ICU). The study protocol was approved by the institutional review board of Oita University Hospital. We obtained informed consent from the patients or their families after explaining the intent of the study.

The study included 15 patients with septic shock who had a SOFA score ≥ 7 at ICU admission (Table 1), were under continuous sedation achieved by artificial respiration with tracheal intubation, and were thought to require nutrition support with a feeding tube for at least 1 week. Septic shock was defined according to the definition of severe sepsis issued jointly by the American Thoracic Society and the Society of Critical Care Medicine in 1992 [17], and patients were treated in accordance with the Surviving Sepsis Campaign guidelines issued in 2008 [18]. Briefly, fluid or blood was transfused until central venous pressure reached 8 to 12 mm Hg, and dopamine, noradrenalin, and vasopressin were administered as needed. If judged necessary by the ICU physician, continuous renal replacement therapy was performed or hydrocortisone was administered. Exclusion criteria were previous use of ANOM, other immune enhancement nutrient product, or immunosuppressive therapy; or diagnosis of acquired immunodeficiency syndrome or malignant tumor.

Content	ANOM (200 mL)
Energy (kcal)	200
Protein (g)	10
Glutamine	1.5
Arginine	0.92
Fat (g)	5.6
Saturated fatty acids	2.86
Medium-chain triglycerides	1.98
N-6 fatty acids	0.58
N-3 fatty acids	0.30
Carbohydrate (g)	27.9
Dietary fiber	1.0
Oligosaccharides	0.48
Water (g)	170.2
Vitamins	
B1 (mg)	0.36
B2 (mg)	0.4
Niacin (mg)	3.2
B6 (mg)	0.60
Folic acid (μg)	76
B12 (μg)	0.64
Biotin (μg)	1.4
Pantothenic acid (mg)	1.9
C (mg)	200
A (μgRE)	140
E (mg)	10
D (μg)	2
K (μg)	16
Minerals	
Sodium (mg)	260
Chlorine (mg)	160
Potassium (mg)	272
Magnesium (mg)	62
Calcium (mg)	126
Phosphorus (mg)	176
Chromium (μg)	12
Molybdenum (μg)	10
Manganese (mg)	0.670
Iron (mg)	1.76
Copper (mg)	0.30
Zinc (mg)	3.0
Selenium (μg)	10
Iodine (μg)	26
DNA (mg)	26
Catechin (mg)	70
Proanthocyanidin (mg)	40

Table 1: Nutritional content of ANOM antioxidant-enriched liquid diet.

After ICU admission, the total caloric intake was supplied intravenously until the patient recovered from shock. After recovery, a feeding tube was placed in the stomach or duodenum, and the continuous infusion of ANOM was initiated at 20 mL/hr. The feeding rate was increased every 8 hours by 10 to 20 mL/hr based on the amount remaining in the stomach and gastrointestinal symptoms (e.g., diarrhea). Intravenous feeding was gradually decreased if appropriate. The target total caloric intake was set at 25 kcal/day, blood glucose level was maintained at 100 to 150 mg/dL, and serum triglycerides were maintained at a normal level.

ANOM is a concentrated liquid diet developed by EN Otsuka Pharmaceutical Co., Ltd. in Japan. ANOM is enriched with antioxidant nutrients and n-3 fatty acids, which suppress the inflammatory response. As shown in Table 1, the caloric content is 20% protein, 55% carbohydrate, and 25% lipid, with a nonprotein calorie/nitrogen ratio of 100. The antioxidant compounds include polyphenols (catechin, proanthocyanidin); high concentrations of vitamin C, vitamin E, and trace elements; and abundant n-3 fatty acids including eicosapentaenoic acid, docosahexaenoic acid, and α-linolenic acid. The ratio of n-6 to n-3 fatty acids is 2:1.

The following parameters were assessed at baseline and after 1 week of ANOM infusion: SOFA score as an index of multiple organ failure [19], platelet count as a marker of vascular endothelial injury [20], serum C-reactive protein (CRP) level as a marker of inflammation [21], PaO2/FiO2 ratio as an index of oxygenation, and total serum bilirubin and creatinine levels.

Measurement of serum 8-OHdG

Radial arterial blood specimens were obtained at baseline and 1 week after ANOM infusion was initiated and centrifuged for 10 min (3500×g, 4°C) to separate the components. The serum was stored at −80°C until analysis, and the level of serum 8-OHdG was determined as a marker of oxidative injury with the Highly Sensitive 8-OHdG Check ELISA kit (normal range: 0.1–0.3 ng/mL) (Nikken SEIL Co., Ltd., Shizuoka, Japan) [22-24].

Electron spin resonance analysis of VCR [15,16]

Radial arterial blood was obtained at baseline and after ANOM infusion was initiated (on days 1, 2, 3, 4, 5, 6, and 7). Blood specimens were centrifuged for 10 min (3500×g, 4°C) to separate serum for the immediate analysis of serum VCR as an indicator of antioxidant potential. Within 2 min after centrifugation, 50 μL serum was transferred to a new light-resistant polyethylene tube, mixed with 50 μL DMSO (Sigma Chemical Co.), and stirred for 10 sec. The samples were transferred to a quartz cell and analyzed by electron spin resonance (ESR) spectroscopy within 1 min after mixing using a highly sensitive ESR spectrometer (JES-FR30; JEOL, Tokyo, Japan). This simple compact ESR system can be used at the patient's bedside, enabling real-time ESR analysis. ESR parameters were set as follows: microwave frequency, 9420 MHz; microwave power, 4 mW; field center, 335.5±5 mT; modulation frequency, 100 kHz; amplitude, 1250; modulation width, 0.079 mT; time constant, 0.1 sec; and sweep time, 2 min. Manganese oxide was used as the internal standard. Manganese oxide powder produces six signals at room temperature; the VCR/DMSO

signal was observed between the third and fourth signals as two peaks with similar intensity, a g-value of 2.005, and a coupling constant aH of 1.8 G. The signal intensity of VCR relative to the third manganese oxide signal was calculated to determine the serum VCR level (Figure 1).

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Baseline values of each parameter were compared with values obtained 1 week after the initiation of AROM infusing by unpaired Student's t-test. The time course of serum VCR changes before and after ANOM administration was analyzed using Fisher's PLSD. P<0.05 was considered significant.

Results

Clinical data

The causes of septic shock in the 15 patients evaluated in this study are listed in Table 2. The most common cause was postoperative complications (acute cholecystitis, n=1; mediastinitis, n=1; disseminated intravascular coagulation, n=1; or acute respiratory distress syndrome, n=2), followed by multiple trauma (n=4).

Patient characteristics are shown in Table 3. The patient population included more men (n=9) than women (n=6). More patients (n=11) received ANOM via the duodenum than via the stomach (n=4). Nine patients received hydrocortisone treatment, and 12 patients underwent continuous renal replacement therapy.

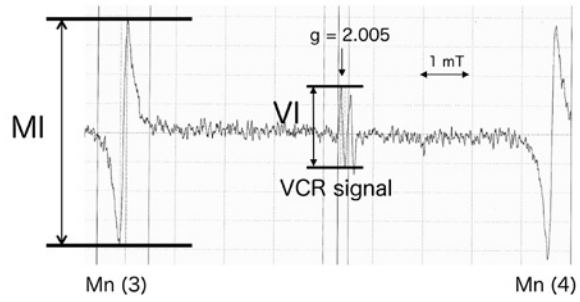

Figure 1: Typical electron spin resonance (ESR) spectra of vitamin C radicals (VCR, ascorbyl radicals) detected in the serum of a healthy subject after DMSO addition. Manganese oxide (MnO) was used as an internal standard. VCR ESR signals were recorded between the third and fourth MnO signals. The intensity of the first VCR signal relative to that of the third MnO signal (VI/MI) was defined as serum VCR.

	Patients (n)
Postoperative complication	
Acute cholecystitis	1
Mediastinitis	1
Disseminated intravascular coagulation	1
Acute respiratory distress syndrome	2
Panperitonitis	2
Severe burn	1
Multiple trauma	4
Heat stroke	1
Toxic epidermal necrolysis	1
Pyoderma gangrenosum	1

Table 2: Etiology of septic shock in patients with multiple organ failure (n=15).

Changes in clinical parameters after ANOM infusion

Clinical parameters at baseline and 1 week after the initiation of ANOM infusion are shown in Table 4. The mean SOFA score after the 1-week AROM administration (6.2±1.7) decreased significantly from baseline (10.9±1.9; p<0.0001), whereas the platelet count (16.1±8.3×104/μL) increased from baseline level (6.3±6.0×104/μL; p=0.0009). Serum CRP level was 9.1±6.6 mg/dL at day 7, which was lower than the baseline level of 21.0±14.0 mg/dL (p=0.0059). Similarly, the post-treatment PaO2/FiO2 ratio (292.8±93.8 mm Hg) improved significantly over baseline (209.4±92.0 mm Hg; p=0.0204). However, total serum bilirubin and creatinine levels at day 7 (5.9±6.7 mg/dL and 1.47±1.42 mg/dL, respectively) did not change significantly from baseline (7.2±8.5 mg/dL and 1.30±0.85 mg/dL). Taken together, our findings show that AROM infusion significantly improved SOFA score, platelet count, serum CRP level, and PaO2/FiO2 ratio compared with baseline, whereas total serum bilirubin and creatinine levels were not changed.

Characteristic	Patients (n=15)
Age, yr (mean)	22–84 (66.7)
Height, cm (mean)	136–178 (159.8)
Weight, kg (mean)	41–77.5 (59.2)
Male/female	9/6
Access route (stomach/duodenal)	4/11
Hydrocortisone (yes/no)	9/6
Continuous renal replacement therapy (yes/no)	12/3
28-day mortality (death/survival)	4/15

Table 3: Patient characteristics.

	Baseline	After treatment	p-value
SOFA score	10.9±1.9	6.2±1.7	<0.0001
Platelet count (104/μL)	6.3±6.0	16.1±8.3	0.0009
C-reactive protein (mg/dL)	21.0±14.0	9.1±6.6	0.0059
Total bilirubin (mg/dL)	7.2±8.5	5.9±6.7	Ns
Creatinine (mg/dL)	1.30±0.85	1.47±1.42	Ns
PaO_2/FiO_2 ratio (mm Hg)	209.4±92.0	292.8±93.8	0.0204

Table 4: Effect of ANOM infusion on organ function in patients with septic shock.

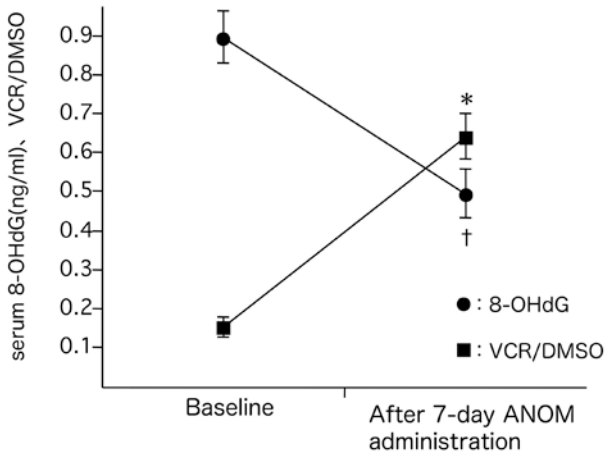

Figure 2: Effect of ANOM infusion on serum 8-hydroxydeoxyguanosine (8-OHdG) and vitamin C radicals (VCR). Square was indicated the signal intensity of VCR/DMSO and circle was indicated the serum concentration of 8-OHdG. Mean ± SD (n=15); * p<0.0001 (baseline VCR vs. after 7-day ANOM administraLon) †p<0.0001 (baseline 8-OHdG vs. after 7-day ANOM administration).

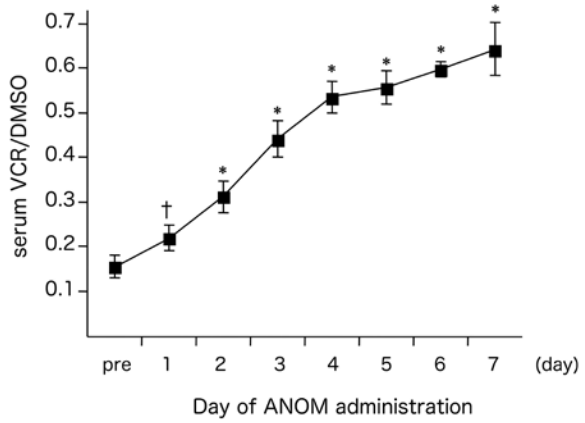

Figure 3: Serum vitamin C radical (VCR) level during the 7-day ANOM administration. Mean ± SD (n=15); †p<0.0001 (compared with previous day) †p=0.0005 (compared with previous day).

Changes in serum oxidative stress levels after ANOM administration

Changes in serum 8-OHdG and VCR levels after ANOM infusion are shown in Figure 2. The mean serum 8-OHdG level decreased from 0.896±0.065 ng/mL at baseline to 0.495±0.061 ng/mL at day 7 ($p<0.0001$), and serum VCR level increased from 0.155±0.026 at baseline to 0.642±0.059 at day 7 ($p<0.0001$).

The time course of changes in serum VCR is shown in Figure 3. The serum VCR level increased daily during the 1-week infusion (baseline, 0.155±0.02; day 1, 60.22±0.029; day 2, 0.312±0.035, day 3, 0.441±0.041, day 4, 0.535±0.035, day 5, 0.555±0.037, day 6, 0.597±0.017, day 7, 0.642±0.059; baseline vs. day 1, $p=0.0005$; other daily increases, $p<0.0001$).

Discussion

In this study, we demonstrated that infusion feeding of the antioxidant-enriched concentrated liquid diet ANOM improved the primary outcomes of oxidative stress and multiple organ failure in patients with septic shock. Furthermore, ANOM appeared to suppress vascular endothelial injury and the inflammatory response, as evidenced by an increased platelet count and reduced serum CRP level.

A mouse study previously reported the superior antioxidant properties of ANOM compared with standard enteral nutrients [25]. After a 7-day diet of ANOM, mice were challenged with lipopolysaccharide (5 mg/kg, intravenous injection). Compared with mice that did not receive ANOM, ANOM-treated mice had significantly higher 8-day survival rates, significantly lower lipid peroxide levels, and significantly higher radical scavenging capacity, as determined by the 1,1-diphenyl-2-picrylhydrazyl and 2,2-azinobis-(3-ethylbenzthiazoline sulphonic acid assays [25]. The present study demonstrates that ANOM is able to significantly reduce oxidative stress and ameliorate inflammation, oxygenation, and vascular endothelial injury, even when administered after the onset of septic shock. Our findings indicate the potential of ANOM as a therapeutic tool to treat sepsis and sepsis-related organ injury.

The antioxidative components of ANOM include polyphenols, copper, zinc, and vitamin C. A recent study suggested the usefulness of polyphenols for treating sepsis [26]. In addition, many reports have reported the efficacy of the water-soluble antioxidant vitamin C for treating sepsis [5,13,28-31]. Although copper and zinc, which bind to the superoxide dismutase active center, are essential for maintaining the antioxidant system, these trace elements have not been studied clinically in septic shock patients [27]. In this study, we demonstrated that ANOM significantly reduced serum 8-OHdG levels, demonstrating that these antioxidative components ameliorated oxidative stress during septic shock.

Approximately 1.5 g vitamin C can be stored in the body [32], and humans cannot activate gulonolactone oxidase to synthesize this vitamin. Consequently, septic shock rapidly depletes the body's store of vitamin C unless sufficient amounts are supplemented. Vitamin C is the systemic antioxidant that responds most rapidly to excessive ROS/RNS levels; therefore, its blood concentration is depleted first [12]. We developed a method for real-time determination of serum vitamin C concentrations by measuring serum VCR in previous studies [15,16], which demonstrated a significant positive correlation between vitamin C levels determined by high-performance liquid chromatography and those determined by ESR. We therefore used ESR to determine ROS/RNS and vitamin C levels in the present study. All patients with septic shock exhibited low levels of VCR at baseline (Figure 3). VCR increased immediately at the start of ANOM infusion but required 3 days to return to normal levels. This finding may be relevant to the treatment of excessive oxidative stress in the acute phase of septic shock. Combining intravenous vitamin C with ANOM during this stage may be needed to restore vitamin C levels to the normal range more rapidly. In addition, real-time monitoring may be useful to ensure that excessive amounts of vitamin C are not administered, which may improve prognosis.

In this study, serum 8-OHdG was evaluated as a marker of oxidative injury. When DNA is exposed to ROS, the carbon in position 8 of guanine is oxidized to produce 8-OHdG. During the process of DNA repair, 8-OHdG is released from cells into the blood for urinary excretion. Because 8-OHdG is not metabolized by the body, it is a relatively stable substance, making it a sensitive biomarker for ROS/RNS-induced oxidative injury. Studies have reported that serum 8-OHdG, which is normally present in trace amounts, is elevated in various diseases [22-24]. In this study, we observed that the elevated 8-OHdG levels in septic shock patients were attenuated by day 7 of ANOM administration, as shown in Figure 2.

There are several limitations of this study worth noting. First, we evaluated only 15 patients in this small-scale study. Second, the observation period was only 7 days long. Therefore, evaluation of long-term outcomes of patients receiving ANOM (e.g., mortality rate) is needed. Third, this study was not a controlled clinical trial; therefore, our findings need to be confirmed by a randomized controlled study. We plan to conduct future studies to elucidate these concerns.

In conclusion, the antioxidant-enriched liquid diet ANOM appears to restore depleted vitamin C to normal levels and alleviate oxidative stress, suggesting it may prevent multiple organ failure in patients with septic shock. The Highly Sensitive 8-OHdG Check ELISA kit allows the analysis of serum 8-OHdG rather than urinary 8-OHdG, and ESR analysis of serum VCR can be used to monitor vitamin C levels in real time, which may provide useful parameters to assess the efficacy of ANOM and other antioxidant therapies.

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