Effects of Parenteral Amino Acid Administration on the Postoperative Nutritional Status and Wound Healing of Protein-Malnourished Rats

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Summary In Japan, parenteral nutrition (PN) solutions are frequently administered to patients in the postoperative short-term period. In these cases, amino acid-containing peripheral parenteral nutrition (PPN) solutions, amino acid-free maintenance solutions or combinations of the two are used. However, consensus regarding the most beneficial solution for these patients is lacking. Here, we examined the nutritional status and wound healing outcomes in protein-malnourished rats receiving postoperative administrations of PPN solution, maintenance solution or combinations of the two solutions. Protein malnutrition was induced in Sprague-Dawley rats by feeding an AIN-93G-based low-protein diet (5% casein) for 2 wk. After laparotomy, dorsal skin incision, and placement of a jugular vein catheter, the rats were divided into 3 groups. Each group was administered 113 kcal/kg/d, with group A receiving maintenance solutions without amino acid, group B receiving PPN with 1.5% amino acid, and group C receiving PPN with 3% amino acid. After 5 d post-operative administration, we measured the tensile strength of the wound area, skeletal muscle weights, and nutritional parameters. Significantly higher plasma nutritional parameters and gastrocnemius and extensor digitorum longus (EDL) muscle weights were observed in groups B and C than in group A. Group C exhibited significantly elevated tensile strength of the wound area along with up-regulation of type I collagen mRNA expression compared to group A. These findings demonstrate the nutritional status and wound healing benefits of short-term postoperative administration of PPN solutions containing amino acids in protein-malnourished rats.

Key Words PPN solution, parenteral nutrition, amino acids, wound healing, protein malnutrition

In Japan, peripheral parenteral nutrition (PPN) is generally used for patients in the short-term period after surgery (1, 2), and during the transition to oral intake or enteral nutrition (EN) therapy. Most commercial PPN solutions contain 3% amino acids, 7.5% dextrose, and electrolytes. However, for short-term postoperative fluid therapy such as 3–5 d, maintenance solutions containing 2.5–10% dextrose and electrolytes without amino acids are sometimes used solely instead of the PPN solutions or combinations of the two.

During the perioperative period, malnutrition can influence patient outcomes such as morbidity, mortality, length of stay, infection rates, and wound healing (3, 4). Garwe et al. (5) examined geriatric trauma patients who were admitted to the emergency department and found that 65% had protein-energy malnutrition (PEM). In addition, these patients had higher risk of in-hospital complications. Furthermore, other previous studies have determined that malnutrition during the perioperative period was a risk factor for delay in wound healing (6, 7). A study investigating early administration of EN during the postoperative period showed improved wound healing in these patients (8). However, there are some cases where patients who cannot use the gastrointestinal tract are unable to receive EN. Even if EN is feasible, it is difficult to satisfy their energy requirements by EN alone. Therefore, parenteral nutrition (PN) is indicated in these situations. However, there are few studies that have investigated changes in wound healing with administration of PPN solutions immediately after surgery.

Even for a short term, the effects of amino acids in the PPN solutions should be commonly recognized among clinicians. Thus, the current study examined the nutritional status and wound healing outcomes in protein-malnourished rats receiving postoperative administrations of PPN solution, maintenance solution or combinations of the two.

MATERIALS AND METHODS

Animals. Male 5-wk-old Sprague-Dawley rats were purchased from Charles River Japan (Yokohama, Japan). All rats were housed under constant temperature (23±3°C) and humidity (55±15%) conditions dur-
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Table 1. Composition of parenteral nutrient solutions (/1,000 mL) and daily dosage.

| Composition  | A       | B       | C       |
|-------------|---------|---------|---------|
| Dextrose (g) | 100     | 87.5    | 75      |
| Amino acid (g) | 0      | 15      | 30      |
| NPC/N       | 0       | 149     | 64      |
| Total energy (kcal) | 400   | 410     | 420     |
| Daily dosage|         |         |         |
| Volume (mL/kg/d) | 284  | 277     | 270     |
| Total energy (kcal/kg/d) | 113  | 113     | 113     |
| Dextrose (g/kg/d) | 28.3 | 24.2    | 20.3    |
| Amino acid (g/kg/d) | 0    | 4.1     | 8.1     |

NPC/N: non-protein calories/nitrogen ratio.

Table 2. Amino acid composition of PPN solution (BFLUID).

| Amino acids     | g/1,000 mL |
|----------------|-----------|
| 1-Leucine      | 4.20      |
| 1-Isoleucine   | 2.40      |
| 1-Valine       | 2.40      |
| 1-Lysine       | 3.15      |
| 1-Threonine    | 1.71      |
| 1-Tryptophan   | 0.60      |
| 1-Methionine   | 1.17      |
| 1-Cysteine     | 0.30      |
| 1-Phenylalanine| 2.10      |
| 1-Tyrosine     | 0.15      |
| 1-Arginine     | 3.15      |
| 1-Histidine    | 1.50      |
| 1-Alanine      | 2.40      |
| 1-Proline      | 1.50      |
| 1-Serine       | 0.90      |
| Glycine        | 1.77      |
| 1-Aspartic acid| 0.30      |
| 1-Glutamic acid| 0.30      |
| Total amino acids | 30.0     |
| BCAA content rate | 30% w/w   |

Experimental design and operative procedures. After 12 d of adaptation, rats were fed an AIN-93G-based low-protein diet (5% casein) or standard AIN-93G (20% casein) diet for 2 wk. The rats fed the low-protein diet (protein-malnourished rats) were divided into three groups, namely, A (n = 12), B (n = 10), and C (n = 11). Rats fed the standard AIN-93G diet were used as the control group D (n = 6). All the animals of each group were fasted overnight at the end of 2 wk of feeding. Next, all rats underwent a 4-cm midline laparotomy and 4-cm dorsal skin incision while under sodium pentobarbital anesthesia. Rats in groups A–C received a silicon catheter that was placed in the jugular vein of each animal. After the operation, all rats were placed in individual metabolic cages for 5 d. Immediately after the surgery, rats in groups A–C were administered different nutrient solutions, and were deprived of both food and water. The control rats in group D were provided with ad libitum access to the standard AIN-93G diet and water. The control rats in group D were provided with ad libitum access to the standard AIN-93G diet and water. The control rats in group D were provided with ad libitum access to the standard AIN-93G diet and water. This group was set as the target baseline as it mimicked the nutritional parameters of persons who do not have PEM before surgery and can eat normal meals sufficiently after surgery.

Parenteral nutrient solutions. A commercial maintenance solution containing 10% dextrose and electrolytes (Physio35; Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) and a commercial PPN solution containing 3% amino acids, 7.5% dextrose and electrolytes (BFLUID; Otsuka Pharmaceutical Factory, Inc.) were used. Rats in group A received Physio35 (0% amino acids), whereas rats in group B received a mixed solution that contained equal volumes of Physio35 and BFLUID (1.5% amino acids); rats in group C received BFLUID (3% amino acids). Table 1 lists the composition of each nutrient solution and the daily dosage. Rats in group A–C were provided 113 kcal/kg/d of each nutrient solution. This dosage corresponds to about 2,000 mL/d (840 kcal/d) of commercial PPN solution that is currently used in persons weighing 50 kg. This dosage was based on a previous study about PPN-infused rats, which indicated that the calorie intake of 120 kcal/kg/d for rats corresponds to calorie intake of 1,000 kcal/d in a person weighing 60 kg (9). Table 2 lists the amino acid composition of PPN solution (BFLUID).

Measurements of nutritional parameters. Body weights were measured prior to surgery (day 0) and at the end of the study (day 5). On day 5, rats were anesthetized using intravenous injection of sodium thiopental. After the collection and processing of blood from an abdominal vein, plasma samples were stored at −80°C until analysis. Plasma levels of albumin, total protein, and blood urea nitrogen were measured using the Hitachi 7180 Biochemistry Automatic Analyzer (Hitachi High-Technologies Corp., Tokyo, Japan). The rapid-turnover proteins, prealbumin (transthyretin) and transferrin, were analyzed using ELISA kits (Abnova Corporation, Taipei, Taiwan and Aviva Systems Biology Corporation, San Diego, CA) according to the manufacturer’s protocol. In all animals, the gastrocnemius, soleus, and EDL muscles were excised and weighed.

Wound healing assay. During the surgery, the dorsal skin incision site was closed using a surgical stapler. Five days after the initial surgery, the dorsal skin incision area was harvested, and the surgical staples were removed. The tensile strength of the skin wound was measured using a Digital Force Gauge ZP-500N (Imada Inc., Aichi, Japan) to evaluate wound healing.

Quantitative real-time PCR. After measurement of the tensile strength of the dorsal skin incision site, samples that contained the incised and closed sites were
frozen in liquid nitrogen and stored at −80°C until RNA extraction. Total RNA was isolated using the PureLink® RNA mini kit (Life Technologies, Carlsbad, CA). Extracted total RNA was reverse transcribed into cDNA using ReverTra Ace® qPCR RT Master Mix with the Thunderbird SYBR qPCR mix (Toyobo). The primer sets used included forward: 5′-ATGGTGGTGAAGACGCCAGTA-3′, Reverse: 5′-GGCACAGTCAAGGCTGAGAATG-3′, and Forward: 5′-TCCACATGCTTTATTCCAGCAATC-3′, Reverse: 5′-GAAGGCCAGCTGTACATCAAGGA-3′ (Takara Bio Inc., Otsu, Japan). The mRNA expression was quantified using the comparative ΔCT method, with Gapdh as the reference gene. The mRNA expression levels were expressed relative to the mean expression observed in group A.

**Plasma amino acid analysis.** Plasma samples were diluted with water and deproteinized using acetonitrile. Subsequently, all the samples were centrifuged and the supernatants were collected. The amino acid concentrations in the supernatants were analyzed by LC/MS/MS using the Acquity UPLC I Class with Xevo TQ-S system (Waters Corporation, Milford, MA).

**Statistical analysis.** All data represent means±standard deviation (SD). The statistical significance among all of the groups compared with group D was assessed using one-way ANOVA followed by Dunnett’s test. The statistical significance among the three nutrient solution groups was assessed using one-way ANOVA followed by the Tukey-Kramer test. All statistical tests were performed using the EXSUS Version 7.7.1 software (CAC Croit Corporation, Tokyo, Japan). Values of p<0.05 were considered statistically significant.

**RESULTS**

**Nutritional parameters**

Table 3 lists the nutritional parameters, which include the body weight and plasma biochemical parameters. After receiving the low-protein diet for 2 wk, the body weight of rats of groups A, B, and C fell significantly (initial body weight) compared to the weight of group D rats. After the 5 d infusion period for each of the nutrient solutions, the final body weight of group C rats increased significantly compared to those of group A and B. Levels of albumin and total protein in group A rats and the body weight gain for the group B and C rats increased significantly compared to those of group A rats. Levels of albumin and total protein in group A were significantly lower compared to those of group D; in addition, the levels in the group B and C rats were significantly higher than that observed for the rats in group A.

**Plasma amino acid analysis.** After receiving the low-protein diet for 2 wk, the body weight of rats of groups A, B, and C fell significantly (initial body weight) compared to the weight of group D rats. After the 5 d infusion period for each of the nutrient solutions, the final body weight of group C rats increased significantly compared to those of group A rats. Levels of albumin and total protein in group A were significantly lower compared to those of group D; in addition, the levels in the group B and C rats were significantly higher than that observed for the rats in group A. The blood urea nitrogen levels gradually increased in rats ingested amino acid dose in each group. Evaluations of the plasma rapid-turnover protein levels showed that
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the concentrations of prealbumin in group A were significantly lower than that observed in the group D rats. Prealbumin levels were significantly higher in group C rats than in group A rats. Transferrin levels were significantly higher in the group B and C rats than in group A rats.

Skeletal muscle weight

After 2 wk on the low-protein diet and 5 d administration of the nutrient solutions, there was a significant reduction in skeletal muscle weights for the gastrocnemius, soleus, and extensor digitorum longus (EDL) muscles of the rats in groups A, B, and C compared to those in the group D rats. In addition, a significant increase in the weights of the gastrocnemius and EDL muscles were observed in the group B and C rats compared to those of the group A rats (Table 4).

Tensile strength of the dorsal skin incision site (wound healing assay)

We performed a wound healing assay to evaluate the effect of each of the nutrient solutions on wound healing in protein-malnourished rats. As seen in Fig. 1, the tensile strength of the dorsal skin wound area in group C rats was almost identical to that observed for group D rats, whereas it was significantly elevated compared to those in group A rats.

mRNA expression levels of type I and type III collagen

We analyzed the mRNA expression of wound healing markers (type I and type III collagen) in the dorsal skin incision area to investigate the potential mechanisms of wound healing. As seen in Fig. 2A, there was a significant up-regulation of the type I collagen (Col1a1) mRNA expression in group C compared to that in group A. Figure 2B shows that there was a tendency for up-regulation of the type III collagen (Col3a1) mRNA expression in group C compared to that of group A (p<0.1).

Profile of plasma amino acid levels

Table 5 presents the plasma amino acid profiles. Compared to the other groups, group A exhibited significantly increased levels of plasma glutamine, glutamic acid, and alanine. After administration of the nutrient solutions in groups A–C, we observed infused amino acid concentration-dependent increases in the plasma arginine, isoleucine, leucine, lysine, phenylalanine, tryptophan, and valine concentrations. These amino acids were all included in the PPN solution used in this study.
DISCUSSION

PEM is one of the factors that can delay wound healing (6, 7). In contrast, appropriate nutritional support improves nutritional status and wound healing. The present study examined the effect of immediate short-term postoperative administrations of PPN solution with amino acids or maintenance solution without amino acids on nutritional status and wound healing in protein-malnourished rats.

Our initial findings showed that the feeding of a low-protein diet for 2 wk induced a significant reduction of the overall body weight. We examined the effect of administering each solution immediately after surgery in these protein-malnourished animals. At the end of the study, our findings demonstrated that rats administered PPN solutions containing 1.5% or 3% amino acids exhibited improved nutritional status, such as a body weight gain, and increase in the plasma levels of albumin and total protein. Moreover, the levels of the rapid-turnover proteins such as prealbumin and transferrin increased in these groups. Previous studies have shown that plasma rapid-turnover proteins have a short half-life and thus are more precise nutritional markers than albumin during the acute phase (10, 11). Indeed, Jiménez Jiménez et al. (12) examined the clinical use of PPN solutions and reported that a 5-d infusion of PPN solutions immediately after surgery improved nitrogen balance and levels of prealbumin, retinol-binding protein, and 3-methylhistidine compared to the baseline values of the surgical patients.

Our study also showed that a 5-d administration of PPN solutions containing 1.5% and 3% amino acids increased the weights of the gastrocnemius and EDL muscles of rats. Studies in humans and animals show that branched-chain amino acids (BCAA), especially leucine, stimulate muscle protein synthesis (13, 14). And Sugawara et al. (15) reported the inhibitory effect of leucine on muscle protein degradation in low-protein diet-fed (5% casein) rats. They showed that addition of leucine to low protein diet suppressed the decrease in gastrocnemius muscle weight and the release of 3-methylhistidine from EDL muscle. Since the PPN solution used in this study was BFLUID, which is a BCAA- (30% w/w BCAA of total amino acids) and leucine-enriched total parenteral nutrition (TPN) solutions improved the nutritional status and reduced the postoperative complications in malnourished surgical patients with gastrointestinal cancer.

Our study also evaluated the tensile strength of the

| Table 5. Profile of plasma amino acid levels (nmol/mL). |
|------------------------------------------------------------------|
| **A** | **B** | **C** | **D** |
| --- | --- | --- | --- |
| Alanine | 515.2±78.5* | 282.1±53.0f | 267.9±34.5f | 261.3±56.1 |
| Arginine | 157.4±43.6 | 303.5±24.6# | 367.3±82.1## | 200.2±30.0 |
| Asparagine | 35.9±4.0f | 22.8±5.2f | 19.2±3.0f | 27.7±5.0 |
| Aspartic acid | 10.5±3.0f | 7.5±0.8f | 7.5±1.0f | 6.8±0.7 |
| Citrulline | 27.9±4.6f | 32.3±6.2f | 34.9±4.6f# | 52.7±4.2 |
| Cysteine | 18.9±2.6 | 22.7±1.9# | 22.6±2.9# | 17.2±2.7 |
| Glutamic acid | 43.1±10.6f | 29.2±8.2f | 26.0±6.0f | 28.5±3.7 |
| Glutamine | 7,396.6±467.9* | 5,757.4±374.7f | 5,350.5±321.8f | 5,764.4±359.4 |
| Glycine | 186.1±27.7 | 227.2±29.2f | 233.6±31.2f | 200.1±30.5 |
| Histidine | 95.4±8.7 | 91.8±6.2 | 127.9±116.0 | 67.1±9.4 |
| Hydroxyproline | 18.0±2.8 | 18.3±2.3 | 19.4±3.7 | 17.9±4.1 |
| Isoleucine | 47.1±6.3 | 60.9±9.5 | 88.5±51.5# | 77.8±5.0 |
| Leucine | 82.4±10.7* | 114.2±18.1 | 165.1±60.8f† | 128.2±11.4 |
| Lysine | 548.3±80.8* | 763.8±110.8# | 969.4±214.6#7 | 820.5±167.4 |
| Methionine | 26.8±5.5f | 43.9±6.3# | 49.2±4.6f# | 20.3±3.8 |
| Ornithine | 150.5±71.0 | 134.7±23.1 | 429.5±363.6 | 99.5±24.3 |
| Phenylalanine | 48.6±7.0 | 77.0±12.2 | 96.4±46.6f# | 54.4±7.1 |
| Proline | 157.1±14.8f | 159.8±19.5f | 187.7±51.3f | 262.9±92.0 |
| Serine | 335.0±56.8f | 208.5±31.4## | 178.0±20.7## | 85.7±21.1 |
| Threonine | 140.1±28.8 | 419.5±70.4f | 343.0±110.1## | 108.1±35.2 |
| Tryptophan | 52.3±16.2f | 96.6±11.7# | 118.1±19.7## | 79.1±7.8 |
| Tyrosine | 29.4±7.4 | 40.3±9.5f | 41.2±3.9f | 34.3±6.8 |
| Valine | 95.2±12.4* | 132.6±21.1* | 211.0±70.8# | 183.6±16.8 |

Results represent means±SD. * indicates p<0.05 vs. group D among all experiment groups by Dunnett’s test. # indicates p<0.05 vs. group A and † indicates p<0.05 vs. group B among three nutrient solution groups by the Tukey-Kramer test. (A: n=12, B: n=10, C: n=11, D: n=6)
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...dorsal skin wound area and demonstrated that PPN solutions containing 3% amino acids promoted wound healing. To the best of our knowledge, this is the first experimental animal study to demonstrate that postoperative administration of PPN solutions not only improves the nutritional status but also the wound healing outcomes. In another recent study involving animal experiments, Gündoğdu et al. (18) reported that preoperative oral nutritional intervention improved anastomotic wound healing in calorically-restricted rats. In our current study, we found that the postoperative administration of PPN solutions containing 3% amino acids promoted wound healing in protein-malnourished rats. Thus, the administration of PPN solutions containing amino acids immediately after surgery would be efficacious for wound healing. Furthermore, the significant increase in type I collagen and a tendency for increase in type III collagen mRNA expression indicate that these factors might play a part in the mechanism that underlies the promotion of wound healing. Type I and type III collagens have been shown to play crucial roles in wound healing (19). Oishi et al. (20) reported that 8 d of a protein-free diet induced down-regulation of the type I and type III collagen mRNA expression in the dorsal skin of rats. This result suggests that protein malnutrition can deteriorate skin conditions. The wound healing process consists of 3 consecutive phases, namely, the inflammatory phase, proliferative phase, and remodeling phase. Type III collagen is produced in the proliferative phase, and thereafter, collagen replacement from type III to type I occurs in the remodeling phase (21). Thus, the differences in the collagen mRNA expression levels observed between groups C and D may have contributed to this process. Moreover, it should be noted that the tensile strength of the wound area for these groups was similar. However, with respect to collagen mRNA expression levels, the wound healing of group D rats with a normal nutritional state was faster than that of the group C rats in which type III collagen was replaced by type I collagen.

Our analysis of plasma amino acids revealed changes in the levels of various amino acids. Glutamine, glutamic acid, and alanine concentrations were especially elevated in the group that was administered the maintenance solutions without amino acids. Since muscle tissues contain large amounts of these amino acids (22), the significant reduction in muscle weight indicates that amino acids from the degraded muscle were released into the blood. Moreover, we also observed that the concentrations of the plasma arginine, isoleucine, leucine, valine, lysine, phenylalanine, and tryptophan were higher in the group that was administered the PPN solutions containing 3% amino acids. Murakami et al. (23) reported that the administration of a combination of BCAAs and glutamine stimulated dermal collagen protein synthesis in protein-malnourished rats. In addition, Zandifar et al. (24) reported that arginine administration improved wound healing in diabetic rats. Overall, these previous findings are partially consistent with our results and speculation that the elevation of these plasma amino acid levels is associated with promotion of wound healing.

However, our current study is not without limitations. First, we estimated type I and type III collagen mRNA expressions only 5 d after the post-operative administration of PPN to investigate the mechanisms underlying the promotion of wound healing. However, the wound healing process is very complex (21), and thus, other factors may be involved in wound healing and other time points may be required. Second, the energy intake between the groups administered with the nutrient solutions (A–C), which were given equal amounts of energy, and the control group rats, who could consume experimental diet ad libitum, might have varied. However, since our objective was to investigate the effects of the nutrient solutions on nutritional status and wound healing, we believe that the conditions used to evaluate the postoperative administration of nutrient solutions with or without amino acids are appropriate. Third, we used young rats in this study. However, the majority of patients with PEM are elderly individuals (5). Therefore, further studies using aged rats are required to better mimic the clinical conditions of elderly patients with PEM.

In conclusion, our results suggest that even short-term postoperative administration of PPN solutions containing amino acids increases skeletal muscle weight, improves nutritional status, and promotes wound healing. Therefore, amino acids in the PPN solutions are efficacious, and the use of PPN solutions within a clinical setting might be a valuable asset in the recovery of protein-malnourished patients.

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Authorship

AW and DH contributed to the conception and design of the study. AW, CS, YM, YH, and AN contributed to the acquisition of data, analysis, and interpretation of the data. AW drafted the article. AW and DH critically revised the manuscript. All authors gave final approval for the final manuscript.

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