Effects of Lipid Emulsion and Multivitamins on the Growth of Microorganisms in Peripheral Parenteral Nutrition Solutions

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Abstract

Background: Blood stream infections caused by Bacillus cereus or Serratia marcescens in patients receiving peripheral parenteral nutrition (PPN) have occasionally been reported in Japan, but these microorganisms are not major causes of blood stream infections in patients receiving total parenteral nutrition via a central venous catheter. In Japan, commercially available PPN solutions contain amino acids, glucose, and electrolytes, but not contain lipid emulsion (LE) and multivitamins (MV). In this study, the effects of LE and MV on the growth of microorganisms such as Bacillus cereus, Serratia marcescens, Staphylococcus aureus, and Candida albicans in PPN solutions were investigated. Methods: A commercial 3% amino acid and 7.5% glucose solution with electrolytes (AF) was used as the base solution to prepare test solutions (LAF, AFV, and LAFV) containing LE, MV, or both. Specifically, 20% LE was added to AF in a ratio of 1:9 to prepare LAF. MV was added to AF and LAF to prepare AFV and LAFV, respectively. A specified number of each microorganism was added to each 100 mL of AF, LAF, AFV, and LAFV in sterile plastic flasks, and all flasks were allowed to stand at room temperature. The number of colony forming units per mL of each microorganism was counted at 0, 24, and 48 hours after the addition of each microorganism. Results: Both Bacillus cereus and Serratia marcescens increased rapidly in AF as well as in LAF, AFV, and LAFV. Staphylococcus aureus did not increase in AF, but increased slightly in LAF and increased rapidly in AFV and LAFV. Candida albicans increased slightly in AF and increased rapidly in LAF, AFV, and LAFV. Conclusions: The results suggest the followings: 1) Bacillus cereus and Serratia marcescens can grow rapidly in PPN solutions consisting of amino acids, glucose and electrolytes; 2) Staphylococcus aureus cannot grow without LE and MV, but can grow rapidly with MV; 3) Candida albicans can grow slowly without LE and MV, and the addition of LE or MV accelerates its growth.

Key words: microbial growth, parenteral nutrition, lipid emulsion, multivitamins, PPN, BSI.

INTRODUCTION

Peripheral parenteral nutrition (PPN) is regarded as a preferred alternative to short-term total parenteral nutrition via central vein (TPN) for many patients, because PPN avoids problems with central venous catheterization, simplifies nursing care, and reduces cost.1,2 In Japan, PPN is usually indicated for patients who require 1-week or shorter parenteral nutrition, and commercial PPN solutions contain...
amino acids, glucose, and electrolytes, but not contain lipid emulsion (LE) and multivitamins (MV). Since conventional short peripheral catheters are commonly used for PPN and midline catheters or fine-bore long catheters are rarely used in Japan, the pH values of commercial PPN solutions are designed to be greater than 6.5 to prevent infusion phlebitis. In other words, such physiological pH of PPN solutions are inevitably suitable for bacterial growth and PPN solutions may become a growth medium for bacterial species more than acidic TPN solutions. Recently, blood stream infection (BSI) outbreaks due to *Bacillus cereus* or *Serratia marcescens* in patients receiving PPN have occasionally been reported in Japan, but these microorganisms are not major causes of catheter-related blood stream infections (CRBSI) observed in TPN patients. In contrast, BSI due to gram positive cocci such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, which are most frequent causes of CRBSI in TPN patients, has rarely been reported in PPN patients. In the present study, we investigated the effect of adding LE or/and MV to PPN solutions on the growth of the microorganisms; ie, *Bacillus cereus* as a delegate of gram positive rods, *Serratia marcescens* as a delegate of gram negative rods, *Staphylococcus aureus* as a delegate of gram positive cocci, and *Candida albicans* as a delegate of fungi.

**MATERIALS AND METHODS**

**Microorganisms employed**

A standard American Type Culture Collection (ATCC) strain and 2 clinical isolates were used for each microorganism, which were the standard strain ATCC11778 and the clinical isolates H-1 and H-2 of *Bacillus cereus*, the standard strain ATCC13880 and the clinical isolates N-4 and N-5 of *Serratia marcescens*, the standard strain ATCC6538 and the clinical isolates N-2 and N-3 of *Staphylococcus aureus*, and the standard strain ATCC10231 and the clinical isolates N-6 and N-7 of *Candida albicans*.

**Test solutions**

A commercial 3% amino acid and 7.5% glucose solution with electrolytes (AF: AMINOFLUID, Otsuka Pharmaceutical Factory, Inc., Japan), a commercial 20% LE preparation (Intralipos 20%, Otsuka Pharmaceutical Factory, Inc.), and a commercial MV preparation (Otsuka MV Injection, Otsuka Pharmaceutical Factory, Inc.) were used. The compositions of AF, Intralipos 20%, and Otsuka MV Injection are shown in Table 1, 2, and 3, respectively. Test solutions (LAF, AFV, and LAFV) were prepared as follows. AF was used as the base solution, and 20% LE was added to AF in a ratio of 1:9 to prepare LAF. To prepare AFV and LAFV, 1/20 of daily dose of MV was added to 100 mL of AF and LAF, respectively.

**Table 1. Composition of AF (AMINOFLUID).**

| Composition per 1000 mL | Characteristics |
|------------------------|-----------------|
|                        | pH | Titratable acidity | Osmolality | OPR |
| L-Leucine              | 4.20 g | 13 mEq | 856 mOs/mL kg | | 6.6 |
| L-Isoleucine           | 2.40 g | 5 mEq | 5 mmol | | |
| L-Valine               | 2.40 g | 20 mEq | | | |
| L-Lysine HCl           | 3.93 g | 5 mEq | | | |
| (as L-Lysine)          | (3.15 g) | 35 mEq | | | |
| L-Threonine            | 1.71 g | 10 mmol | | | |
| L-Tryptophan           | 0.60 g | 5 mmol | | | |
| L-Methionine           | 1.17 g | 5 mEq | | | |
| L-Cysteine             | 0.30 g | 13 mEq | | | |
| L-Phenylalanine        | 2.10 g | 5 mEq | | | |
| L-Tyrosine             | 0.15 g | 6 mEq | | | |
| L-Arginine             | 3.15 g | 20 mEq | | | |
| L-Histidine            | 1.50 g | 10 mmol | | | |
| L-Alanine              | 2.40 g | 5 mmol | | | |
| L-Proline              | 1.50 g | 10 mmol | | | |
| L-Serine               | 0.90 g | 5 mmol | | | |
| Glycine                | 1.77 g | 10 mmol | | | |
| L-Aspartic acid        | 0.30 g | 10 mmol | | | |
| L-Glutamic acid        | 0.30 g | 10 mmol | | | |

*, including the amount derived from the additives. OPR, osmotic pressure ratio to physiological saline.
Table 2. Composition of Intralipos 20%.

| Composition per 100 mL |
|-----------------------|
| Soybean oil          | 20.0 g |
| Egg yolk lecithin    | 1.2 g  |
| Glycerol             | 2.2 g  |

Characteristics

| pH         | 7.2 |
| Osmolality | 284 mOsm/kg |

Table 3. Composition of Otsuka MV Injection.

| Composition per 4 mL (daily dose) |
|-----------------------------------|
| Vitamin B1                        | 3.1 mg |
| Vitamin B2                        | 3.6 mg |
| Vitamin B3                        | 4.0 mg |
| Vitamin B12                       | 0.005 mg |
| Vitamin C                         | 100 mg |
| Folic acid                        | 0.4 mg |
| Nicotinamide                      | 40 mg  |
| Biotin                            | 0.06 mg |
| Panthenol                         | 14 mg  |

Vitamin A oil 3300 Vit. A IU

Vitamin D3 0.005 mg

Vitamin E 10 mg

Vitamin K 2 mg

Addition of microorganism, incubation, and sampling

A specified number of each test microorganism was added to each 100 mL of test solutions in sterile plastic flasks, and all flasks were allowed to stand at room temperature (23-26°C). An aliquot (3-25 mL) of test solution was sampled at 0, 24, and 48 hours after the addition of microorganism. The aliquot for 0 hour was sampled a few minutes after the addition of microorganism, and the values obtained at 0 hour show the added amount of each microorganism.

Measurement of viable cells

Each aliquot of test solution sampled was inoculated in a Soybean Casein Digest (SCD) agar plate in duplicate. When necessary, the test solution sampled was diluted 10-fold to 10^{-7}-fold with physiological saline before inoculation. When 1 to 10 mL of test solution was examined, the solution was filtered with a membrane filter (0.45 μm, nitrocellulose) for the sterility test of Japanese Pharmacopoeia, and the filter was put on a SCD agar plate. After 12 to 24 hours of incubation at 37°C, the number of colony forming units (CFU) of each microorganism was counted for each plate, and the mean CFU of duplicate data was calculated. The number of CFU per mL was calculated by using the number of CFU per plate, inoculated volume, and diluting ratio. The results are shown as values of CFU/mL in semi-logarithmic graphs. Same as other experimental studies of microbial growth, the data obtained in this study were not analyzed statistically because biological significance of these kinds of data is considered assessable without statistical analysis.

RESULTS

Bacillus cereus (Figure 1)

All 3 strains of B. cereus increased rapidly in all of AF, LAF, AFV, and LAFV, reaching the maximum concentration within 48 hours. At 24 hours, the addition of LE or MV accelerated the proliferation.

Serratia marcescens (Figure 2)

All 3 strains of S. marcescens increased rapidly in all test solutions, AF, LAF, AFV, and LAFV, reaching the maximum concentration within 48 hours. At 24 hours, the addition of LE accelerated the proliferation.

Staphylococcus aureus (Figure 3)

In AF, all 3 strains of S. aureus did not increase for 48 hours, and 2 clinical isolates were not visible after 24 hours. In LAF, a clinical isolate N-2 did not increase, while the other 2 strains increased slightly. Also in AFV and LAFV, N-2 did not increase, while the other 2 strains (the standard strain ATCC6538 and the clinical isolate N-3) increased rapidly in AFV and LAFV and almost reached the maximum concentration.

Candida albicans (Figure 4)

All 3 strains of C. albicans increased slightly in AF, but increased rapidly in LAF, AFV, and LAFV.

DISCUSSION

Catheter-related blood stream infection (CRBSI) is one of the most common complications of intravenous catheters. For microorganisms, Staphylococcus aureus, coagulase-negative staphylococci, Candida albicans, Escherichia coli, Klebsiella pneumonia, etc. have been shown as the major causes of CRBSI in patients receiving total parenteral nutrition via central vein (TPN). Recently, blood stream infection (BSI) outbreaks caused by Bacillus cereus or Serratia marcescens in patients receiving peripheral parenteral nutrition (PPN) have occasionally been reported in Japan, but these microorganisms are not major causes of CRBSI in TPN patients. In contrast, BSI due to gram positive cocci such as Staphylococcus aureus or Staphylococcus epidermidis has rarely been reported in PPN patients. In the present study, we investigated the possibility of growth of microorganisms such as Ba-
cillus cereus, Serratia marcescens, Staphylococcus aureus, and Candida albicans in PPN solutions. In particular, the effects of the addition of lipid emulsion (LE) and multivitamins (MV) to the PPN solutions on the microbial growth were investigated.

**Bacillus cereus**

![Graph showing the effect of lipid emulsion or multivitamins on the growth of Bacillus cereus.](image)

*Figure 1.* Effect of lipid emulsion or/and multivitamins on the growth of *Bacillus cereus* in AF (pH is 6.6 and OPR is approximately 3). LAF was prepared by mixing AF and 20% LE in a ratio of 9:1. AFV or LAFV was prepared by adding 1/20 of daily dose of MV to 100 mL of AF or LAF. OPR, osmotic pressure ratio to physiological saline.

**Serratia marcescens**

![Graph showing the effect of lipid emulsion or multivitamins on the growth of Serratia marcescens.](image)

*Figure 2.* Effect of lipid emulsion or/and multivitamins on the growth of *Serratia marcescens* in AF (pH is 6.6 and OPR is approximately 3). LAF was prepared by mixing AF and 20% LE in a ratio of 9:1. AFV or LAFV was prepared by adding 1/20 of daily dose of MV to 100 mL of AF or LAF. OPR, osmotic pressure ratio to physiological saline.

**Staphylococcus aureus**

![Graph showing the effect of lipid emulsion or multivitamins on the growth of Staphylococcus aureus.](image)

*Figure 3.* Effect of lipid emulsion or/and multivitamins on the growth of *Staphylococcus aureus* in AF (pH is 6.6 and OPR is approximately 3). LAF was prepared by mixing AF and 20% LE in a ratio of 9:1. AFV or LAFV was prepared by adding 1/20 of daily dose of MV to 100 mL of AF or LAF. OPR, osmotic pressure ratio to physiological saline.
In the study, a commercial 3% amino acid and 7.5% glucose solution with electrolytes (AF) was used as the base solution, and AF was added with LE, MV, or both. A specified number of each microorganism was added to each 100 mL of test solutions in sterile plastic flasks. The number of colony forming units (CFU)/mL of each microorganism was counted at 0, 24, and 48 hours after the addition of microorganism. All 3 strains of *B. cereus* increased rapidly in all test solutions, and the addition of LE or MV seemed to accelerate the proliferation. This finding indicates that *B. cereus* can increase rapidly in PPN solutions without LE and MV, and the addition of LE or MV accelerates the growth. All 3 strains of *S. marcescens* increased rapidly in all test solutions, and the addition of LE seemed to accelerate the proliferation. Thus, it is indicated that *S. marcescens* also can increase rapidly in PPN solutions without LE and MV and the addition of LE accelerates the growth. All 3 strains of *S. aureus* did not increase for 48 hours in AF without LE and MV, and 1 clinical isolate did not increase in AF added with LE, MV, or both. However, the other 2 strains increased slightly in AF added with LE, increasing rapidly in AF added with MV and in AF added with both LE and MV. These results indicate that *S. aureus* cannot increase in PPN solutions without LE and MV, but some strains may increase rapidly by adding MV. Since 2 of the 3 strains increased rapidly with no increase in 1 strain, there seems to be a strain difference in auxotrophy of *S. aureus*. All 3 strains of *C. albicans* increased slightly in AF without LE and MV, but increased rapidly in AF added with LE, MV, or both, indicating that *C. albicans* increases slightly in PPN solutions without LE and MV and the addition of LE or MV accelerates the growth. These results show that *B. cereus* and *S. marcescens* can grow rapidly in PPN solutions containing amino acids, glucose and electrolytes, but *S. aureus* cannot grow similarly.

We have previously shown that adding water-soluble vitamins does not affect the growth of *S. aureus* but water-insoluble vitamins are essential for the growth of *S. aureus*.23 PPN solutions available in Japan consist of amino acids, glucose and electrolytes but do not contain LE and MV, although the latest PPN products contain only thiamine (B1) for the prevention of Wernicke’s encephalopathy or lactic acidosis. In clinical practice, PPN solutions are sometimes supplemented with water-soluble vitamins such as B1, B2, B6, B12, and C, but rarely supplemented with MV including water-insoluble vitamins. With regard to the pH value of PPN solutions, the physiological pH of Japanese PPN solutions seems to be suitable for bacterial growth,5,6 and *B. cereus* and *S. marcescens* can increase rapidly without LE and MV. With regard to BSI, cases with *B. cereus* and *S. marcescens* have been reported in PPN patients, while cases with *S. aureus*, perhaps also with *S. epidermidis*, have scarcely been reported in PPN patients. This is likely because those cannot increase without LE and water-insoluble vitamins. On the other hand, commercially available PPN solutions in European countries and some other countries are acidic (pH 5-6)2,24,25 as similar to TPN solutions, and then BSI outbreaks by *B. cereus* or *S. marcescens* have rarely been reported. However, PPN and TPN solutions with physiological pH have recently been launched in European countries,26 and these solutions contain LE and are usually supplemented with MV, which would be suitable for the growth of all the microorganisms employed in the present study.

Taken together, the results of this study suggest...
the following: if microbial contamination occurs, 1) Bacillus cereus and Serratia marcescens can grow rapidly in PPN solutions consisting of amino acids, glucose and electrolytes; 2) Staphylococcus aureus cannot grow without LE and MV, but can grow rapidly with MV; 3) Candida albicans can grow slightly without LE and MV, and the addition of LE or MV accelerates its growth. Therefore, it is strongly recommended that even in PPN, nutrient solutions must be prepared under strict aseptic conditions and procedures, and intravenous lines must be cared properly.

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ABBREVIATIONS
AF: a commercial 3% amino acid and 7.5% glucose solution with electrolytes (AMINOFLUID, Otsuka Pharmaceutical Factory, Inc., Japan); AFV: a test solution prepared by adding 1/20 of daily dose of multivitamins to 100 mL of AF; ATCC: American Type Culture Collection; CFU: colony forming units; CRBSI: catheter-related bloodstream infections; LAF: a test solution prepared by mixing AF and 20% lipid emulsion in a ratio of 9:1; LAFV: a test solution prepared by adding 1/20 of daily dose of multivitamins to 100 mL of LAF; LE: lipid emulsion; MV: multivitamins; OPR: osmotic pressure ratio to physiological saline; PPN: peripheral parenteral nutrition; SCD: Soybean Casein Digest; TPN: total parenteral nutrition via central vein; Vit.A IU: international unit for vitamin A.

COMPETING INTERESTS
TK, SK and KS are employees for Otsuka Pharmaceutical Factory, inc. YI has declared that no competing interest exists.

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