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Original article

Factors influencing *Candida albicans* growth in parenteral nutrition with and without lipid emulsion: Using an established framework to inform maximum duration of infusion policy decisions☆

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SUMMARY

Background & aims: Because lipid putatively encourages contaminant growth it has been proposed that infusion of lipid-containing parenteral nutrition (PN) bags should be restricted to 24 h (48 h or longer if lipid free). This study aimed to examine this proposal by identifying factors affecting *Candida albicans* growth in PN.

Methods: *C. albicans* growth was assessed in quadruplicate in 12 PN infusates, with and without lipid and varying glucose concentrations.

Results: The results are presented as mean ± SEM. Baseline $\log_{10}$ colony forming units (cfu)/mL (1.806 ± 0.015) increased substantially by 48 h in the PN infusates (to 3.731 ± 0.059). In PN infusates (pH 6.14 ± 0.01) growth was unaffected by the presence of 5% w/v lipid (0.246 ± 0.156 $\log_{10}$ cfu/mL decrease; $P = 0.127$), and independently suppressed by increasing glucose concentration (0.438 ± 0.174 $\log_{10}$ cfu/mL decrease per 10% increase in w/v glucose; $P = 0.018$). In a separate analysis growth was suppressed by increasing energy density (0.520 ± 0.179 $\log_{10}$ cfu/mL decrease per 1000 kcal non-nitrogen energy in 2 L; $P = 0.007$), without a significant effect of % non-nitrogen energy from lipid (0.056 ± 0.036 $\log_{10}$ cfu/ml increase per 10%; $P = 0.082$).

Conclusions: Using a framework developed to examine growth of potential contaminants in PN, the inclusion of lipid emulsion in PN produced no specific effect on the growth of *C. albicans*, other than by increasing energy density. Growth was independently suppressed by increasing either glucose concentration or non-nitrogen energy density.

1. Introduction

Parenteral nutrition (PN) is often used to treat patients with temporary or permanent intestinal failure but it is associated with complications, one of the most serious of which is catheter-related sepsis (CRS). CRS can lead to significant morbidity, which delays recovery from illness at considerable associated cost to the health service, and death.

The treatment of CRS due to *Candida albicans* can be difficult, often requiring removal of the intravenous catheter used for PN delivery. The reported rapid growth of *C. albicans* in PN infusates, especially those with lipid (or lipid emulsion alone), has been a cause of added concern. However, concerns about the growth potential of *C. albicans* in PN infusates need to be put into context for three reasons. First, it is unlikely that *C. albicans* will be incorporated into PN bags if they are compounded according to recognised

Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; cfu, colony forming units; CRS, catheter-related sepsis; g, grams; kcal, kilocalorie; L, litre(s); mL, millilitres; mmol, millimoles; mOsm/L, milliosmoles per litre; *P*, probability; PN, parenteral nutrition; SEM, standard error of the mean; % w/v, percent weight in volume (grams per 100 mL).

☆ *Conference presentation*: Part of the work was presented in abstract form at the European Society for Parenteral and Enteral Nutrition annual Congress in Barcelona, Spain, in 2012.

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standards in Europe[1] or the United States[2] (Chapter 797 of the US Pharmacopeia). Second, if PN infusates are filtered through 0.2 μm diameter pores, or 1.2 μm diameter if lipid emulsion is used, according to certain society recommendations[3,4] *C. albicans* will not pass through the filter. Third, *C. albicans* is not usually the most common organism associated with CRS.

If recommendations are to be made about restricting the duration of PN infusions according to the composition of PN infusates, which may influence microbial growth, they should take into account the growth of multiple microorganisms, especially those that are commonly responsible for CRS or less common organisms that grow avidly in PN solutions. While *C. albicans* is not the most common organism associated with CRS it has certainly contributed to guideline recommendations[5–7] about the maximum duration of PN bag infusion because it grows rapidly in PN solutions. In contrast, *Staphylococcus epidermidis* which is one of the most common organisms responsible for CRS in patients being administered PN,[8,9] has often been reported to grow sluggishly in PN infusates. We have investigated the growth potential of *S. epidermidis* in PN infusates of different composition,[10] but did not find support for guidelines' recommendations to restrict infusion of lipid-containing PN bags to 24 h.[5–7] We now turn our attention to investigating the growth potential of *C. albicans* in PN infusates using the same approach. This approach examines whether there is a specific effect of lipid in PN infusates that stimulates the growth of *C. albicans* that dominates and overrides any independent effects of glucose concentration, energy density and pH of the PN.

2. Materials and methods

The growth of *C. albicans* was assessed in lipid emulsion alone and in PN regimens with and without lipid emulsion in the presence of varying glucose concentrations. All PN infusates were prepared in 500 mL multi-layered PN bags (Evarex from B. Braun; Mirandola, Italy). In some cases, the pH of the PN admixtures was adjusted upwards to correspond to the pH of the lipid emulsion alone and *vice versa*. Detailed descriptions of the procedures for preparing the PN regimens, pH adjustment (to within 0.05 units of the target pH where applicable), inoculation, and subsequent sampling and reporting of results are described elsewhere.[10]

In brief, 250 mL fills of different PN regimens (Table 1) in oxygen barrier bags to reflect recommended practice and to reduce the confounding effects of variable oxygen tension were prepared in a validated UK hospital pharmacy aseptic suite and inoculated with *C. albicans* to achieve an initial concentration of approximately 50 colony forming units (cfu)/mL. *C. albicans* National Collection of Pathogenic Fungi (NCPF) 3179 was used (from Health Protection Agency Culture Collections; Salisbury, UK), which is recommended in the British Pharmacopoeia[11] for growth promotion tests. This represents a pathogenic strain, originally isolated from a case of bronchomycosis, and is equivalent to American Type Culture Collection (ATCC) 10231, and to Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures DSM 1386. The bags were stored and sampled (immediately after inoculation and at 24 and 48 h) at a controlled room temperature of 21.5 °C (range during continuing 24 h periods, 20.0–22.3 °C). The sample aliquots were plated on Columbia blood agar prior to incubation at 37 ± 1 °C for 48 h before a blinded operator independently assessed and reported the plate growths, based on two plates at time zero and three plates at both 24 and 48 h.

2.1. Statistical methods

2.1.1. Sample size

To compare two groups (one with and one without a lipid component) a sample size of 16 bags per group is necessary to detect an effect size of 0.38 according to Cohen's criteria[12] (medium effect size, $f = 0.25$ and large effect size, $f = 0.40$) with 80% power and a *P*-value of 0.05.[10]

2.1.2. Statistical analyses

The data were log transformed because they did not conform to a normal distribution. Statistical analyses were undertaken using ANOVA, repeated measures ANOVA and ANCOVA (after confirming the absence of heterogeneity of regression). Unless otherwise stated, the results are presented as mean ± standard error of the mean (SEM). The analyses were undertaken using PASW Statistics package version 19.0 (Chicago, Illinois, USA).

Table 1
The study parenteral nutrition (PN) regimens, all prepared in quadruplicate.

PN regimen		A	B	C	D	E	F	G	H	I
Infusate type		Lipid free PN	Lipid free PN	Lipid free PN	Lipid free PN	Lipid PN	Lipid PN	Lipid PN	Lipid PN	Lipid alone
Volume	mL	2000	2000	2000	2000	2000	2000	2000	2000	2000
Nitrogen[a]	g	9	9	9	9	9	9	9	9	0
Glucose	% w/v	8	11	14	17	8	11	14	17	0
Glucose	kcal	640	880	1120	1360	640	880	1120	1360	0
Lipid[b]	kcal	0	0	0	0	1000	1000	1000	1000	4000
Sodium	mmol	60	60	60	60	60	60	60	60	0
Potassium	mmol	60	60	60	60	60	60	60	60	0
Magnesium	mmol	10	10	10	10	10	10	10	10	0
Calcium	mmol	5	5	5	5	5	5	5	5	0
Phosphate	mmol	25	25	25	25	25	25	25	25	30
Vitamins[c]	mL	5	5	5	5	5	5	5	5	0
Trace elements[d]	mL	10	10	10	10	10	10	10	10	0
Calculated osmolarity	mOsm/L	871	1038	1204	1371	945	1110	1277	1443	270
pH-adjusted	Yes or No	No	Yes[e]	No	No	No	Yes[e]	No	No	Yes[e]
Final pH of bag used unadjusted and (adjusted)	pH as mean ± SEM	6.19 ± 0.01	6.17 ± 0.01 (8.15 ± 0.01)	6.20 ± 0.03	6.17 ± 0.03	6.13 ± 0.01	6.11 ± 0.01 (8.16 ± 0.01)	6.08 ± 0.03	6.10 ± 0.02	8.35 ± 0.03 (6.10 ± 0.02)

[a] Synthamin 17EF (Baxter Healthcare Limited, Norfolk, UK).
[b] 20% w/v Clinoleic (Baxter SA, Lessines, Belgium). The phosphate from the lipid emulsion was included.
[c] As 1 vial of Cernevit (Baxter SA, Lessines, Belgium) in 5 mL Water for Injection.
[d] Additrace (Fresenius Kabi, Halden, Norway).
[e] Prepared in quadruplicate for both unadjusted and pH-adjusted (the pH of the lipid was adjusted to match that of both PN infusates, and *vice versa*).