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Morphological Stability of Fat Particles in All-in-one Parenteral Nutrition

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Abstract

Background "All-in-one" parenteral nutrition is an accepted method of nutritional support in surgical patients. However, controversy still exists in the stability of fat emulsions, when mixed with other nutritional components and hang for a period of infusion. The general consensus is that particle larger than 5 microns should not be administered, since particle larger than 6 microns increase the risk of fat embolism and may be associated with hemodynamic changes. A study was conducted to evaluate the physical stability of fat particles in parenteral nutrition systems currently practiced in pediatric surgical intensive care unit, Siriraj Hospital, to ensure that it is safe for clinical use.

Material and methods Seven admixtures of all-in-one parenteral nutrition, actually given for patients in pediatric surgical ICU, were examined under a transmission electron microscope. Each admixture contained glucose (10.47 - 11g/dl), amino acid (0.7 - 2.0 g/dl), 20% Intralipid, electrolytes and vitamins in a specific amount tailored for one day infusion of an individual patient. Specimens were collected from hanging bottles at immediately, six and twenty-for hours after mixing. The data were analyzed regarding the average sizes of fat particles, amount of large particles per counting area and maximum particle diameters (D-max). Comparisons were made between pure 20 % Intralipid and mixed emulsion. Statistical analysis was evaluated by Student's t-test.

Results Microscopic evidence of instability, such as coalescence was not detected in all samples. Average diameters of fat particles in 20 % Intralipid was 0.418 ± 0.144 microns, whereas particles in admixtures range in size between 0.341 ± 0.152 and 0.595 ± 0.283 microns. The average particle diameter in four of seven immediate samples was significantly larger than particle sizes in pure emulsion. Five admixtures had increased mean particle sizes at the twenty-forth hour post mixing, compared to the immediate solution. At 0, 6th and 24th hour post-mixing, large size (over than 4 microns) were found 0, 0.14 and 0.29 particles per counting area, respectively. No particle larger than 5 microns was detected in all specimens. Maximum diameter of particles (D-max) in pure 20% Intralipid was 1.74 microns whereas D-max in all admixture ranged from 1.3 to 4.65 microns (average 2.50 microns). Average D-max of twenty-forth-hour solution (2.62 microns) are not significantly larger than those of immediate sample (2.01 microns).

Conclusion Fat particles change their morphology slightly,when mixed in an all-in-one system and hang in room temperature for twenty-four hours. However, stability is still in the acceptable range within one day of infusion.

Parenteral nutrition (PN) has become an essential part of pre and post operative care in pediatric surgery. All-in-one parenteral nutrition system is one administration technique in which lipid emulsion is combined together with conventional solution of glucose, amino acids electrolytes and trace minerals in a single container for direct infusion.[1] This method is becoming more widely accepted because of its convenience, by eliminating the need of pumps and joint connectors, thus reducing overall cost and risk of infections.[2,3] Above all, this technique can provide a high caloric dense solution in low osmolality. Therefore, giving complete parenteral nutrition via peripheral veins is, therefore, possible.[1,4] Although complications are uncommon, there remains controversy regarding the stability of fat emulsions when mixed with other components. There is in-vitro evidence that, under inappropriate conditions, fat particles fuse together, forming large particles. Particles larger than 6 microns may cause pulmonary complications or hemodynamic change.[4,5]

The physical stability of fat particles in all-in-one parenteral nutrition, currently practiced in a pediatric surgical intensive care unit was then investigated, to ensure clinical safety.

Materials & Methods

Seven admixtures of all-in-one PN, which were given to patients in the pediatric surgical intensive care unit, Siriraj Hospital were examined by gross visual observation and microscopic examination by transmission electron microscope. Each PN formula was tailored daily for an individual patient, taking into account the patient's weight, clinical conditions and serum electrolytes.

The characteristics of each admixture are listed in Table 1. Glucose concentrations range from 10.47 to 10.89 gram-percent whereas amino acids (10% amino acid N pad, Frezenius, Germany) 1.3 to 2.0 gram-percent. Lipid emulsions consist of soy bean emulsion (20% Intralipid, Kabi Pharmacia AB, Stockholm, Sweden) in amounts of 2-4 grams per one kilogram of patient weight. Solutions were routinely mixed at the ward in the morning, just before infusion. Admixtures were hang at 25°C room temperature during twenty four hours of administration. Gross morphological observation was practiced as standard nursing care throughout the administration period.

Microscopic study was done using electron microscopic technique. Three samples from each admix-

Table I Characteristics of all-in-one parenteral nutrition in the study

Solution no.	1	2	3	4	5	6	7
Total volume (ml)	1528	397.5	413	910	395	368	8285
Dextrose (g%)	10.47	10.82	10.89	10.73	10.89	10.81	10.74
10%aminoacid N pad (g%)	1.3	2.01	1.57	1.9	1.52	1.61	1.45
20%Intralipid (ml/L)	98.2	113.2	96.9	122.3	113.9	161	121
Sodium (mEq/L)	39	42.4	21.8	24.5	22.8	75.4	29
Potassium (mEq/L)	18.3	45	9.7	10.9	15.2	30.2	19.3
Chloride (mEq/L)	39	22.6	21.8	24.5	22.8	75.4	29
Acetate (mEq/L)	24.9	35.2	19.4	21.7	25.3	34.2	24.1
Phosphate (mmol/L)	3.3	5	4.8	5.4	5.1	2	4.8
Calcium (mmol/L)	1.6	3.8	3.6	4.1	4.4	2.5	3.6
Magnesium (mEq/L)	2.6	5	4.8	5.4	5	4	2.4
Addamel (a) (ml)	2	1	0.5	0.5	0.5	2	2
Vitamins (b) (ml)	6	5	5	5	5	4	4
Osmolality (mOsm/Kg)	776	820	765	928	882	1075	945

a Addamel-N (Pharmacia AB, Stockholm, Sweden)

b Vitamins; consist of 1 ml of Vitamin K plus 2 - 4 ml of OMVI (Otsuka Pharmaceutical, Tokyo, Japan) (Case#1,6,7) or 1ml of Vitamin K plus 2 ml of Vitalipid (Pharmacia & Upjohn AB,Stockholm, Sweden) and 2 ml of Soluvit N (Kabi Pharmacia AB, Stockholm, Sweden) (Case #2- 5)

ture were taken at different intervals, immediately after mixing, 6 and 24 hours aftermixing. The specimens were fixed in 4 % glutaraldehyde, buffered with phosphate to pH 7.4. The fluids were spun and the sediments were fixed again in glutaraldehyde and post-fixed in 2% osmium tetroxide in the same buffer. The fixed tissues were then dehydrated with a series of ascending concentration of ethanol and embedded in Epon-812. One micron-thick sections were obtained with an LKB microtome, stained with toluidine blue, and used for selecting appropriate areas for ultra-thin sectioning. Ultrathin sections were also cut and stained with uranyl acetate and lead citrate. The grid were examined and photographed with a-E2L 100 SX electron microscope at 80 kV.

Plates of 7,240 X - magnification were examined regarding microscopic morphology of fat particles. Evidence of instability i.e. fusion of fat cells or cracked granules were to be noted. Large particles (diameter larger than 4 microns) per one unit area (three fields) were counted. Particles of the largest diameter ware measured and recorded as maximum particle diameter of each sample (D-max). All particles in three field of 22,000 X - magnification were measured and average sizes of each sample were then calculated.

Comparisons were then made between pure 20% Intralipid and mixed all-in-one solutions and among samples collected from different hanging durations. Statistical analysis was performed, using Student T - test. Statistical significance is considered with p-value less than 0.05.

Results

Gross visual observation showed no evidence of precipitating, creaming or oiling-out. Microscopically, fat particles in all admixtures are well distributed without any evidence of particle aggregation or fusion. Morphology of particles in all mixed solution are not obviously differed from those of pure lipid emulsion. No cracked or shrunken particles were noted (Figure 1).

The average diameter of fat particles in 20% Intralipid is 0.418 ± 0.144 microns. Average particle diameters of samples collected immediately after mixing range from 0.341 to 0.595 microns (Table 2). Four of seven immediate samples contain significantly larger mean particle sizes than in pure lipid emulsion. When comparing average sizes of samples collected at the twenty-forth hour to the initial specimen, it was found that there were significant increase in five admixtures.

The maximum particle diameters (D-max) of each sample varied from 1.3 to 4.65 microns whereas D-max of 20 % Intralipid is 1.74 microns (Table 2). Average D-max of 24th hour sample (2.62 microns) is not significantly larger than that of immediate admixture (2.01 microns).

Regarding large particle count, at 0, 6th and 24th hour - post mixing, large sized (over than 4 microns) fat particles were found at averages of 0, 0.14 and 0.29 cells per counting area, respectively. No particle larger than 5 microns was observed in all studying specimen.

Discussion

Since its introduction by Solassal in 1972, all-in-one parenteral nutrition system has gained more popularity in post operative nutritional support in intensive care setting, surgical ward or even home parenteral nutrition because of its relative convenience in preparation and administration.[1-5] It has been widely accepted among pediatric surgeon that parenteral nutrition contributes much to successful surgical outcome. Neonates undergoing surgery of gastrointestinal tract usually can not be fed via enteral route for a few weeks or even months. Lipid emulsion mixed in the all-in-one system lessens the overall solution osmolality thus giving high caloric density.[4-6] Therefore, complete parenteral nutrition is possible to be administered via their peripheral veins during post operative starvation period.

Controversy still exists regarding safety in combining emulsions of fat with other components. Actually,[7-9] fat is the most vulnerable component of the solution. Under inappropriate conditions such as low pH, too much cations and/or too long storage time, fat particles may loss their stability.[4-5] Instability begins with aggregation of fat particles which may progress to particle fusion, developing larger particles. Particles larger than 6 microns have been reported to cause adverse reactions, particularly pulmonary complications. Aggregated particles usually move to the surface of the emulsion, forming a cream layer. Creaming is a stage that can be reversed by gentle agitation. If the aggregated particles are left untreated, they will fused together and finally separate as an oily layer, seen at the

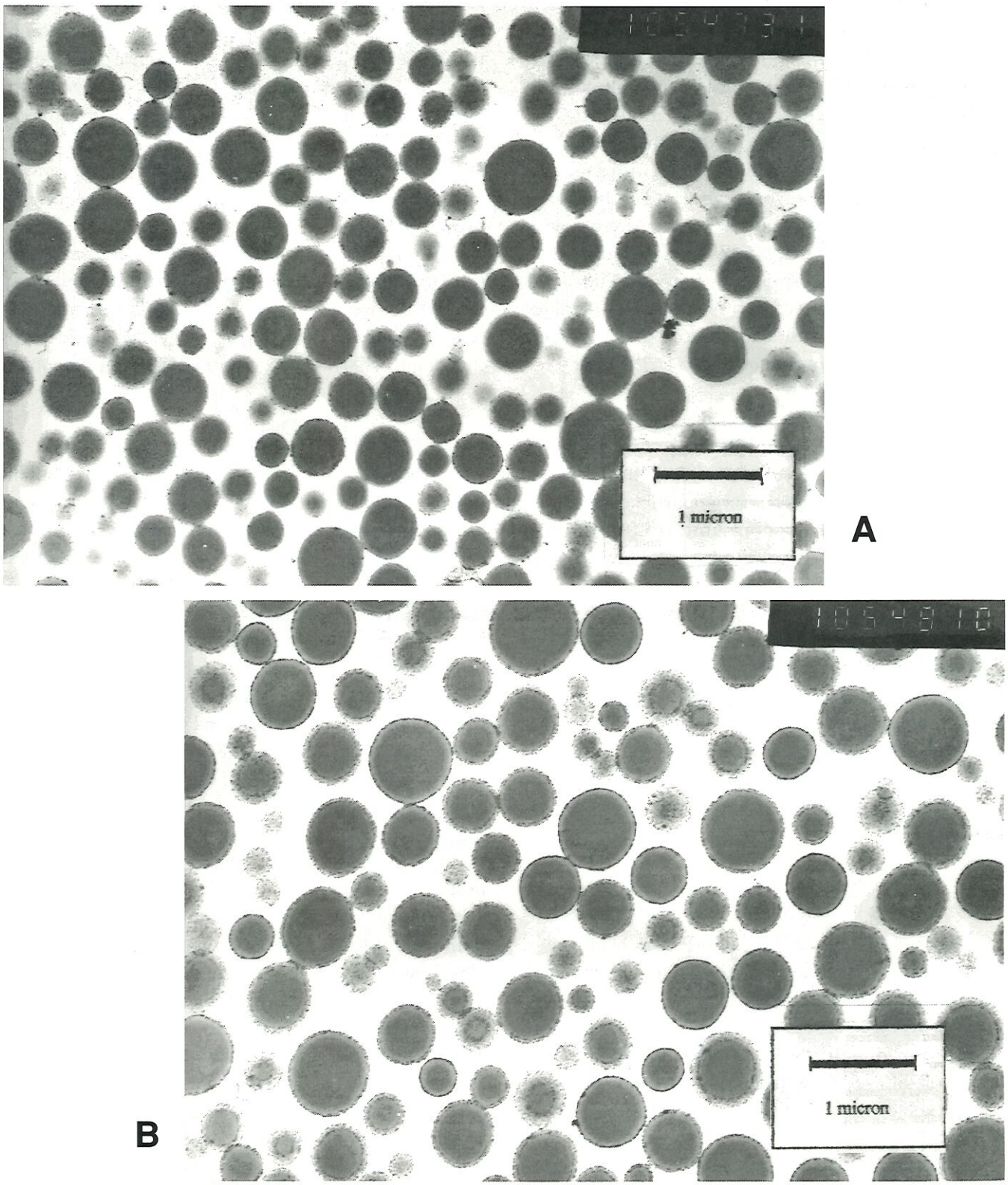

Fig. 1 Microscopic pictures of fat emulsion.

A. 20% Intralipid

B. a 24th hour post mixing sample of all-in-one admixture (X 22,000). Note that the distrbution and particies shape are not significantly different from those of 20% Intralipid.

Table 2 Measurement of fat particles in all-in-one admixtures and count of large particle per three plates of 9,240 X

Admixture no.	Average particle size (microns)			Maximum particle size (microns)			Large particle count (particle/area)		
	Initial	6th-hour	24th-hour	Initial	6th-hour	24th-hour	Initial	6th-hour	24th-hour
1	0.595	0.531	0.506*	2.7	1.84	3.24	0	0	0
2	0.395	0.511	0.509	2.81	3.46**	4.65	0	2	2
3	0.437	0.582	0.503*	1.73	4**	1.4	0	1	0
4	0.341	0.575	0.509*	1.51	3.03	2.59	0	0	0
5	0.361	0.502	0.514	1.95	2.27**	2.6	0	0	0
6	0.419	0.506	0.578	2.06	2.6**	1.73	0	0	0
7	0.542	0.565	0.518*	1.3	3.03	2.16	0	0	0
		Average		2.01	2.89	2.62	0	0.14	0.29

**Significantly larger than average particle size of 20% Intralipid*

***Significantly larger than average particle size of the same bottle at 0-hour (initial solution)*

top of the emulsion. Besides direct visual observation, evaluation of stability can be performed by measurement of particle size and size distribution, microscopic observation and surface potential study. Emulsions that lose their stability have microscopic evidence of aggregation or particle fusion, pathological change of an individual particle, change in particle size as well as size distribution.[4]

Black and Popovich[10] found that concentrated dextrose can deteriorate the lecithin-emulsifying system due to its acidic nature, monovalent cation induced a progressive increase in the rate of globule fusion and divalent cation caused an immediate aggregation of 10% lipid emulsion. Anyway, they also mentioned that the pH-lowering effect of dextrose can be buffered by amino acid solution and combining dextrose - amino acid together with fat emulsions is possible for at least 72 hours. Further study by Pamperl and Kleinberger[11] was carried by mixing 60% glucose, 10% amino acid and 20% lipid emulsions. It was demonstrated by electron microscopic sections that fat particles were damaged by highly concentrated glucose. Various studies have been conducted in "clinically used" all-in-one formulas that possess "not extreme" ranges of dextrose, amino acid or electrolyte solutions and are mixed for a certain infusing duration. Tannuri et al[12] reported that mixing lipid emulsions with dextrose up to 25% concentration, and addition of electrolytes as routinely utilized in parenteral nutrtion, do not cause any damage to fat particles, at least for 24 hours. Sayeed et al[13] evaluated pH, particle size, osmolality and zeta potential of all-in-one admixtures, using various intravenous fat emulsions and amino acids. They found no significant change at one day storage at room temperature or nine day refrigeration at 5°C plus one day at room temperature.

The average size of fat particle in pure 20% Intralipid in this study (0.418 microns) is compatible with previous reports.[14,15] Despite this, there appears to be no evidence of coalescence, the mean particle sizes of admixtures do have slight but significant change, compared to pure emulsions. This increase in particle diameters may be an effect of decreasing zeta potential, caused by the addition of electrolytes or dextrose solution.[4] Moreover, the sizes tend to grow larger over hanging time. Change in average sizes over time goes along with frequency of large particle count, which seems to increase over hanging duration. Nevertheless, particle larger than 5 microns which should not be infused is not detected in all specimen, within 24 hour of routine utilization.

All-in-one parenteral nutrition has been practiced at our pediatric surgical intensive care unit since 1987 with no appreciable adverse effect.[1] The majority of settings are post-operative nutritional support in neonates. This study provide basic scientific data that may legitimize this technique of parenteral nutrition in this patient group. Anyway, the technique is of no side effects at all.[1,4,5] Care must be taken concerning safety range of glucose concentration, mixing consequences and limitation of hanging time. Recommended final dextrose concentration is between 10 - 23 per cent. Electrolytes, trace elements or undiluted dextrose should never be added directly into pure lipid emulsions. Practically, dextrose, aminoacids and

other additives are mixed together in a bottle. Note that calcium should be the last to add to avoid precipitation with phosphate.[8] Lipid emulsion is finally mixed down only if the solution looks clear. At room temperature, the admixture should be administered within 24 hours. Most conservative guidelines for prolonged storage are 7 days at 4°C.[4,5]

Conclusion

Fat particles change their morphology slightly, when mixed in All-in-one PN and hang in room temperature for 24 hours. However, stability is still in accepted range within one day of infusion.

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