Experience of compounding total parenteral nutrition admixtures for preterm infants in a hospital pharmacy: evidence of calcium and phosphate compatibility problem

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ABSTRACT

Objective Parenterally fed preterm newborn infants require large amounts of calcium and phosphate in a low volume of solution. The lower the volume of solution, the higher is the possibility of precipitation of calcium hydrogen phosphate ($CaHPO_4$). Precipitation could cause respiratory distress and pulmonary embolism, and the use of organic salts of calcium and phosphorus may reduce the likelihood of this problem. To date, no previous work on the stability of solutions with organic salts has been published in the literature. This study aims to evaluate the visible precipitation of calcium and phosphorus in total parenteral nutrition solutions.

Methods 20 parenteral nutrition solutions were aseptically prepared in a laminar airflow hood in a clean room. The solutions are intended to facilitate precipitation, with the amino acid ratio below the standard concentration and other parameters also modulated to promote the precipitation of $CaHPO_4$. The solutions contained dextrose, amino acids, calcium gluconate and fructose 1,6-bisphosphate. We did not use lipid emulsion so that we could see all precipitations.

Results No visible precipitation was observed during 4 weeks of observation at 25°C. The only observed event was the change in colour of the solution, which became yellow, maybe because of a Maillard reaction.

Conclusions This study evaluated the compatibility of organic calcium and phosphorus in order to prevent the precipitation of $CaHPO_4$ when preparing total parenteral nutrition solutions. The fact that no precipitation was observed is very significant as it indicates the compatibility of the ions, even though no instrumental analysis was performed.

INTRODUCTION

The American Academy of Pediatrics recommends that, after birth, preterm infants should grow as a normal fetus of the same gestational age.[1] The minimum rate of growth is 15 g/kg/day during mid-trimester and 10 g/kg/day at term.[2] Often this weight gain is not achievable in preterm infants because extrauterine life requires higher energy expenditure than intrauterine life.[2] Breast milk feeding remains the best way to provide nutrition to the newborn infant, but it is not always possible to start breast feeding immediately after the delivery of a preterm infant[2] because coordination of sucking/swallowing and swallowing/breathing does not occur before 32 and 33–34 gestational weeks, respectively.[3]

Therefore, in order to achieve a neonatal growth rate similar to a normal fetus, early parenteral nutrition (PN) should be started immediately after birth.[2] PN is often used in association with minimal enteral feeding; this permits the administration of low volumes of enteral nutrition to the infant. Enteral nutrition stimulates the development of the gastrointestinal tract, thus improving enzymatic activity, hormonal release, blood flow, intestinal mobility and flora.[4]

The Italian Society of Paediatrics[5] has established that the initial energy requirement of preterm infants is 45–65 kcal/kg/day with a protein:energy ratio of 1:25. Table 1 (modified from table 1 published by the Italian Society of Paediatrics[5]) shows the initial and final requirements of preterm infants. As can be seen from the table, large amounts of calcium (Ca^{2+}) and phosphate $(PO_4)^{3-}$ are needed as these two ions are necessary for the mineralisation of bone. The optimal Ca^{2+}:P ratio is 1.7:1,[6] which is the same as the ratio found in human breast milk.

The problem is intensified by the fact that preterm infants often suffer from oliguria. In such cases, the volume of PN administered should be as low as possible, but the lower the volume, the higher is the risk of precipitation of calcium hydrogen phosphate ($CaHPO_4$).

In 1994, after two cases of death due to pulmonary embolism and two cases of pulmonary distress developed during peripheral infusion of all-in-one total parenteral nutrition (TPN) admixtures, the Food and Drug Administration (FDA) published a public safety alert.[7] According to the FDA, the following parameters should be verified prior to administering TPN:

- pH
- Ca^{2+} and $(PO_4)^{3-}$ concentration and their solubility, which should be calculated from the volume at the time the Ca^{2+} is added
- Phosphate content of amino acid solution
- Order of mixing
- Filtration when infusing
- Visual inspection during the mixing process and before infusing
- Time of administration: if stored at room temperature the infusion should start within 24 hours after mixing; if stored at refrigerated temperatures the infusion should start within 24 hours after being warmed.

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Table 1 Nutritional requirements (macronutrients, minerals and electrolytes) in preterm newborns receiving total parenteral nutrition

	Initial dose	Target dose
Volume (ml/kg/day)	70–90	150
Energy intake (kcal/kg/day)	45–65	120–140
Macronutrients		
Proteins (g)	2–3	3.5–4.2
Protein:energy ratio (g/100 kcal)	1:25	1:25
Carbohydrates (g)	6–7	12–14
Lipids (g)	1–2	4
Minerals		
Calcium (mg/kg/day)	25–50	80–100
Phosphorus (mg/kg/day)	20–30	60–80
Magnesium (mg/kg/day)	2–5	7–9
Electrolytes		
Sodium (mmol/kg/day)	0–1	3–7
Potassium (mmol/kg/day)	0–1	2–3
Chlorine (mmol/kg/day)	0–1	3–5

pH plays a very important role. The solubility of calcium dihydrogen phosphate ($Ca(H_2PO_4)_2$) is 1.8 g/L while the solubility of $CaHPO_4$ is 0.03 g/L. According to the Henderson–Hasselback equation, at pH 7.4, 60% of phosphate is in the bibasic form, $(HPO_4)^{2-}$, which is less soluble. By reducing the pH by 2 units, 95% of phosphate is in the monobasic form, $(H_2PO_4)^-$, which is far more soluble.[8]

The concentration of amino acids is the factor that most influences the pH of the final admixture. Amino acids are able to buffer the solution, depending on the concentration of arginine, histidine and lysine.[9] Raising the final concentration of amino acid in the admixture increases the buffering capacity of amino acids.[10–12] The minimum concentration usually used in paediatric PN is 2.5–3.5%. This value represents the required concentration to exert an effective buffering effect in the TPN admixture.

Lenz and Milkrut[12] underlined the importance of cysteine in reducing the pH of the admixture, with a stabilising effect. In a solution without cysteine, with 2.5% of amino acids and 10 mmol/L calcium chloride ($CaCl_2$), the maximum amount of inorganic phosphate as Na_2PO_4 is 7.5 mmol/L. In admixtures with cysteine (50 mg/dL), the compatible quantity of Ca^{2+} and phosphate rises to 10 mmol/L, thus reducing the medium Z potential of the particles.[13]

In addition to the buffering effect, amino acids can chelate Ca^{2+}. In this way, amino acids can reduce the free rate of the cation.[14] Amino acids such as glutamic acid, aspartic acid, arginine, histidine and lysine are able to bind the ion Ca^{2+}.[15]

Even the amount of dextrose affects the pH of the final solution, but to a less significant extent.[16]

The FDA recommends that an admixture should be administered within 24 hours after warming.[7] Controlling and storing the admixture at a regulated temperature helps to prevent the precipitation of $CaHPO_4$. In fact, the temperature is able to influence the dissociation of the calcium salt used, often Ca^{2+} gluconate, thus increasing the free rate of Ca^{2+} in the admixture.[17]

Increasing the temperature enhances the quantity of the phosphate ion in the monobasic form. Thus, the concentration of $(HPO_4)^{2-}$ increases, being less soluble, while the concentration of the monobasic form $(H_2PO_4)^-$ decreases, being more soluble.[15]

Also, the order of adding Ca^{2+} and phosphorus (P) is very important and must follow a particular order. P must be added to the bag and vigorously mixed before adding Ca^{2+}.[9] Di Salvo[14] suggested adding Ca^{2+} and P to different solutions: Ca^{2+} should be added to the solution of amino acids in order to decrease the free rate of the ion, while P should be added to the hypertonic dextrose solution that promotes the formation of monobasic phosphate, which is more soluble than the bibasic form. However, the infusion of two bags will increase the number of manipulations required when administering the solution, increasing the risk of contamination.[18]

The choice of salt used plays an important role in the stability of TPN admixtures. $CaCl_2$ is the inorganic source of Ca^{2+}. Given that $CaCl_2$ is more dissociated than the organic salt (Ca^{2+} gluconate), $CaCl_2$ reduces the compatibility of Ca^{2+} and P because of a mayor concentration of free Ca^{2+}.[19] Because of this, it is suggested that an organic Ca^{2+} salt such as Ca^{2+} gluconate should be used. The anion gluconate has a larger steric effect than Cl^-, reducing the dissociation constant of the salt. This effect limits the quantity of free Ca^{2+} in the admixture.

With regard to phosphate salts, there are commercially available organic sources of P such as glucose-1-phosphate, glycerol-3-phosphate and fructose-1,6-bisphosphate (FDP). All have a phosphoester bond, which makes the P organic.

FDP is a physiological metabolite of glycolysis[20] and can also be used in cases of hypophosphataemia during transfusion therapies, extracorporeal circulation, chronic alcoholism, lung failure and prolonged malnutrition. This phosphate group donor is easier to handle than inorganic ions because it has better compatibility with Ca^{2+}.[21]

In addition, FDP has the following benefits in vivo:[15]

- It skips three steps of glycolysis, saving two molecules of ATP.
- It keeps a high level of 2,3-bisphosphoglyceric acid in the red blood cells, which enhances the release of oxygen to the peripheral tissues.
- It is slowly eliminated from the kidneys.

The last parameter analysed is the final concentration of bivalent ions such as Mg^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} in the admixture. Schuetz and King[22] suggest that the Mg^{2+} ion is able to influence the likelihood of precipitation because it forms relatively more soluble and stable salts with phosphate ions. Jimenez-Torres and Ronchera-Oms[16] indicate that ratios of Ca^{2+}:Mg^{2+} <2 exert a positive effect on $CaHPO_4$ solubility.

Our analysis focuses on the compatibility of organic Ca^{2+} as gluconate and P as FDP, since so far we have not found any studies evaluating the compatibility of these ions in organic form in TPN admixtures. The scope was to evaluate the concentration of organic Ca^{2+} and organic P that would show evidence of a visible precipitation in the solution. We produced admixtures with a fixed concentration of amino acids of 2%, not beyond the fixed minimum standard concentration of 2.5%. In a second experiment, we changed factors such as dextrose, organic–inorganic salt and the concentration of bivalent ions in order to determine which factors are capable of facilitating the precipitation of $CaHPO_4$.

METHODS

TPN admixtures were prepared aseptically following international recommendations under a laminar airflow hood in a clean room at the Total Parenteral Nutrition Laboratory of ASST Bergamo Est Hospital. Ethyl vinyl acetate (EVA) bags (Aries) were manually prepared in order to analyse the compatibility of Ca^{2+} and P. No lipids were added because they would obscure the presence of a precipitate.

We prepared the first four bags using TPH 6% (Baxter) as this product represents the pattern of amino acids in the preterm baby. Because of a low concentration of TPH 6%, we could not standardise the solution to 100 mL with high volumes of Ca^{2+} and P solutions. After a comparison of the composition of TPH 6% and Sintamin 10% (Fresenius Kabi), we decided to change to Sintamin 10% because of its higher concentration of amino acids. As shown in table 2, Sintamin 10% allowed us to reach the standard 2% of amino acids while keeping the standard volume of 100 mL. Despite the fact that TPH 6% contains amino acids such as aspartate, glutamic acid, tyrosine and taurine, the composition of Sintamin is quite similar. Indeed, as shown by Driscoll *et al*,[9] the content of Ca^{2+}-binding amino acids (arginine, histidine and lysine) is equivalent. Even the cysteine composition is similar between TPH 6% and Sintamin 10%; TPH contains 0.33% of the sulfur amino acid while Sintamin contains 0.32%. As stated earlier, cysteine is able to influence the pH and, consequently, the compatibility of Ca^{2+} and phosphate.[14]

After this evaluation we decided to use Sintamin 10% in a final concentration of 2%, which is lower than the standard range of amino acids (2.5–3.5%), in order to promote the precipitation of $CaHPO_4$.

We analysed the compatibility of the organic salts of Ca^{2+} and P using Ca^{2+} gluconate 10% (0.446 mEq/mL; Monico) and FDP 10% (0.47 mEq/mL of P; Esafosfina, Biomedica Foscama). However, bags P15, P16 and P17 contain $CaCl_2$ 10% (1.36 mEq/mL; Monico) because we wanted to reach a higher Ca^{2+} concentration with a lower Ca^{2+} solution volume in the same final 100 mL.

Our TPN solutions were prepared with the aim of containing 2% of amino acids, 10% of dextrose (from dextrose 50%; Baxter). We then tried to add Mg^{2+} (Mg sulfate 0.5 mmol/mL; Monico). To analyse the compatibility with more bivalent ions, we also added Zn^{2+} to the admixtures, alone or together with Mg^{2+}.

Table 2 Comparison between Sintamin 10% and TPH 6%

	Sintamin 10%	TPH 6%
Alanine	6.68	5.32
Arginine	9.02	12.14
Asparagine		
Aspartic acid		3.16
Cysteine	0.32	0.33
Glycine	10.96	3.66
Glutamic acid		4.99
Glutamine		
Isoleucine	6.57	8.15
Histidine	3.14	4.82
Leucine	8.58	13.97
Lysine	16.65	8.14
Methionine	5.10	3.33
Phenylalanine	5.35	4.82
Proline	10.58	6.82
Serine	5.57	3.82
Taurine		0.25
Tyrosine	3.78	4.16
Threonine	3.78	4.16
Tryptophan	1.45	2.00
Valine	6.24	7.81

Table 3 First four tests of the study (P1–P4) with TPH 6%

	P1	P2	P3	P4
Water for injections				
Calcium gluconate 10% (mEq)	24.98	24.98	24.98	24.98
Fructose 1,6-bisphosphate 10% (mEq P)	2.0	2.0	4.0	4.7
TPH 6% (%)	1.98	1.75	1.69	1.69
Dextrose 50% (g)		10	10	9
Final volume (mL)	100	113.26	117.52	117
Precipitation	No	No	No	No
Transparency	Yes	Yes	Yes	Yes
pH	<5.4	4	5	5

Table 4 Next five tests of the study (P5–P9) with Sintamin 10%

	P5	P6	P7	P8	P9
Water for injections (mL)			5		
Calcium gluconate 10% (mEq)	20.07	24.98	12.21	24.98	24.97
Fructose 1,6-bisphosphate 10% (mEq P)	9.87	6.0	9.97	4.68	2.20
Sintamin 10% (%)	2	2	2	2	
Dextrose 50% (g)	7	6	10		10
Final volume (mL)	100	100.77	100.3	100	100
Precipitation	No	No	No	No	No
Transparency	Yes	Yes	Yes	Yes	Yes
pH	5	5	5	5	5

The details of the first four bags (P1–P4) are shown in table 3. TPH 6% was used but, as can be seen from the table, the volume was too high. P1 does not contain dextrose in order to evaluate how sugar affects the stability and compatibility.

Table 4 shows the details of the next five bags (P5–P9). From P5 we started to use Sintamin 10%. The concentration of dextrose was 10% in all the bags, except for P8 which was prepared without dextrose. In order to analyse the effect of amino acids, all bags contained 2% of amino acids, except for P9 which was produced without Sintamin 10%. The variations between P8 and P9 are to evaluate how dextrose and amino acids can influence the precipitation of $CaHPO_4$. Details of the last 11 bags (P10–P20) are shown in table 5. These admixtures contain Mg^{2+} in a Ca^{2+}:Mg^{2+} rate of >2. This rate reduces the compatible concentrations of Ca^{2+} and P, as shown by Boulet and Marier.[21] Also, a bag without dextrose (P14) and a bag without amino acids (P13) were prepared to evaluate the effect of dextrose and amino acids. To reach a higher concentration of Ca^{2+} in the bag while maintaining the final volume at 100 mL, we made three bags with $CaCl_2$ (P15, P16 and P17), as shown in table 5. As this is an inorganic salt, we thought that its higher dissociation rate would facilitate the precipitation of $CaHPO_4$, thus enabling us to compare our work. The last three bags (P18–P20) also contained Zn^{2+} in order to increase the quantity of bivalent ions and analyse their effect on the stability of the admixtures (table 5).

RESULTS

We evaluated immediate and late precipitation. No visible precipitation occurred in either the late or immediate evaluation. A limitation of our study is the absence of instrumental analysis

Table 5 Last 11 tests of the study (P15, P16 and P17 were made with $CaCl_2$)

	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20
Water for injection (mL)	21					32					
Ca^{2+} gluconate 10% (mEq)	9.8	20.3	15.2	25	24.8				13.2	13.2	15.4
$CaCl_2$ (mEq)						24.5	24.5	20			
Fructose 1,6-bisphosphate 10% (mEq P)	6	6	9.96	11.3	11.3	11.8	5.9	5.9	9.6	9.6	9.6
Sintamin 10% (%)	2	2	2		2		2	2	2	2	2
Dextrose 50% (g)	10	8.5	10	7.5		10		10	10	10	10
$MgSO_4$ 1 mEq/mL (mEq)	5	5	5	5	5	5	5	2	5		
$ZnSO_4$ (mg) 1 mg/mL (mg)									5	10	5
Final volume (mL)	100.8	100.8	100.3	100	100	100	100	100	100	100	100
Precipitation	No	No	No	No	No	No	No	No	No	No	No
Transparency	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
pH	5	5	5	5	5	5	5	5	5	5	5

because we do not have a particle counting machine or high-performance liquid chromatography (HPLC). However, this limitation does not lower the reliability of the results because we have produced our bags following our standard operative procedures for preparing sterile TPN admixtures. We produced 20 bags according to our normal method of production before being administered to a patient.

In order to consider the stability of our admixtures, we also checked the pH of each bag. The pH was <5.4; at this value 95% of phosphate is in the monobasic form as $H_2PO_4^-$, which is more soluble.[9]

The bags were kept for 4 weeks at a temperature of 25°C. No precipitation occurred during this period, and the pH did not change. The only observed event was a change in the colour of the solution, which became yellow, possibly because of the Maillard reaction between the amino acids and dextrose.

DISCUSSION

The aim of this study was to evaluate the compatible amount of organic Ca^{2+} and P in TPN admixtures because, to date, there has been no study of the use of these two organic salts in the literature. High Ca^{2+} and high P admixtures were produced to check whether precipitation occurred. Even though we changed many parameters that might make the admixture unstable, $CaHPO_4$ precipitation was not observed.

This study suggests that the use of organic sources of Ca^{2+} and P enhances the compatibility of the two ions. In order to confirm this hypothesis more evaluations are needed, such as a potential Z analysis or a particle count. It would be interesting to analyse a solution of the same composition using a particle counting machine and HPLC. The next step in our analysis is to evaluate the compatibility of Ca^{2+} and lipid emulsions.

What this paper adds

What is already known on this subject?
The compatibility of calcium and phosphorus in total parenteral nutrition admixtures is a well-known problem for pharmacists. Many parameters influence this compatibility, and a number of studies have been undertaken to evaluate the stability of solutions containing inorganic calcium and phosphorus.

What this study adds
This study evaluated the compatibility (and therefore the stability) of total parenteral admixtures containing organic calcium (calcium gluconate) and organic phosphorus (fructose 1,6-bisphosphate). The aim of the study is to evaluate the stability of admixtures containing high concentrations of calcium and phosphorus and to check how some parameters influence the stability of total parenteral nutrition bags.

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Contributors DZ and SL conceived the study and compounded the admixtures.

Competing interests None declared.

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