

Articolo originale - Original article

Fructose-1,6-diphosphate can improve long term stability of home parenteral nutrition (HPN) admixtures

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ABSTRACT: Aim. To evaluate the stability and compatibility of Fructose-1,6-diphosphate within a number of typical Home Parenteral Nutrition regimens in order to avoid Calcium Phosphate precipitation.

Methods. A variety of regimens based on different commercially available parenteral nutrition products Synthamin[®] 14, Vamin[®] 9, Clinomel[®] and Compleven[®] were studied. Calcium gluconate (10%), Esafosfina[®] (Fructose-1,6-diphosphate 7.5%), Addiphos[®] or Potassium Hydrogen Phosphate (17.42%) were added to each admixture to provide a maximum concentration per litre of 22 mmol Calcium and 55 mmol inorganic or organic phosphate. Each combination was thoroughly mixed, inspected, then stored in the dark at room temperature and submitted to visual inspection at 1h, 24h, 4d and 30d. pH was also determined.

Results. No precipitation was observed throughout the study period in any of the Fructose-1,6-diphosphate containing regimens. In contrast supplementary addition of both preparations of inorganic phosphate immediately created turbidity or gross precipitation that remained unchanged.

Conclusions. Our results confirm that at high concentrations of Calcium and Phosphate precipitation occurs when supplementary inorganic phosphate is added to Home Parenteral Nutrition admixtures. The same regimens are stable for up to 30d when organic Fructose-1,6-diphosphate is used and more clinically appropriate Calcium Phosphate ratios of approximately 1:2 are feasible. (RINPE 2001; 19: 144-51)

KEY WORDS: Fructose-1,6-diphosphate, Home Parenteral Nutrition, Calcium Phosphate precipitation

PAROLE CHIAVE: Fruttosio 1,6 difosfato, Nutrizione Parenterale Domiciliare, Precipitazione di calcio-fosforo

INTRODUCTION

Home Parenteral Nutrition (HPN) is now well-established and is often the treatment of choice for adults and children with severe inflammatory bowel disease (IBD), but metabolic bone disease and hypophosphataemia are still experienced by many patients (1, 2). Patients with Crohn's Disease and/or short bowel syndrome (SBS) are at significantly increased risk of osteopenia, osteoporosis and occasional bone fractures. Growth

failure and abnormal bone mineral density are especially prevalent in pediatric patients. The etiologies are multifactorial but Calcium (Ca) and Phosphorus (P) deficiencies play a major role. There is a definite correlation between daily Ca intake and degree of osteopenia in children receiving long-term TPN (3). A high Ca and P content in HPN admixtures can stabilise serum 1,25-dihydroxycholecalciferol and tubular reabsorption of P without increasing urinary Ca excretion.

Nevertheless, a significantly common problem fa-

cing the compounding pharmacist when formulating HPN regimens, is the difficulty associated with the successful avoidance of calcium phosphate (CaP) precipitation. (4) The fact that Ca and P can precipitate as CaP when mixed in the same Parenteral Nutrition (PN) solution is a well documented incompatibility (5). Special attention was brought to this problem by a safety alert issued in 1994 by the US Food and Drug Administration (FDA), (6) which warned of the serious hazard to human health with respect to the supply of P and Ca within PN admixtures by identifying two deaths, both caused by microvascular pulmonary emboli (found to contain a CaP complex). Although the incorporation of normal Ca and P requirements is usually achievable in stable adult regimens it can prove impossible for paediatric and neonatal mixtures when using the standard inorganic sources currently licensed for use in the UK and USA. In other countries, such as Italy, where organic compounds are routinely available, this problem is less common.

A recent, major pharmaceutical study confirms that Fructose-1,6-diphosphate (FDP) has superior stability in comparison to dibasic sodium phosphate in Freamine III[®], (Clintec, Montargis, France) containing PN admixtures over a range of pH, temperature, calcium chloride and amino acid concentrations. (7) Mixtures containing up to 16.7mmol Ca and 30mmol P per litre were stable using FDP, thus allowing recommended daily requirements of both electrolytes to be safely administered. This could not be achieved with inorganic phosphate.

The aim of this study is to further evaluate the stability of calcium (as gluconate salt) with inorganic phosphate (IP) or FDP in three adult HPN and one pediatric HPN admixture.

MATERIALS AND METHODS

Synthamin[®] 14 (Baxter Healthcare, Deerfield Illinois, USA) or Vamin[®] 9 (Fresenius Kabi, Bad Homburg, Germany) were combined in equal volumes with Glucose 50% (Baxter Healthcare, Deerfield Illinois, USA) and electrolytes, then split into three sealed glass containers. Calcium gluconate 10% (Laboratoire Aguettant, Lyon, France) and FDP 7.5% (Esafosfina[®], Biomedica Foscoma, Rome, Italy) were added to each admixture to provide: 2 mmol Ca: 4 mmol P (SI, VI) 10 mmol Ca: 20 mmol P (SII, VII) or 20 mmol Ca: 40 mmol P (SIII, VIII) per L. The two non-lipid compartments of the multichamber bag Clinomel[®] N6-900 (Baxter Healthcare, Deerfield Illinois, USA) (which already provides 2.25 mmol Ca and 15 mmol IP per L) were combined, thoroughly mixed then transferred into four sealed glass

containers (CI, CII, CIII and CIV). A total of 10 mmol Ca/L was added to CI, CII, CIII, CIV as gluconate. Potassium hydrogen phosphate (dibasic potassium phosphate) 17.42% (IP) (Martindale, Romford, UK) was then added to CI (22 mmol P) and CII (55 mmol P) and FDP was added to CIII (22 mmol P) and CIV (55 mmol P). Each combination was thoroughly mixed, inspected, then stored in the dark at RT and submitted to visual inspection at 1h, 24h, 4d and 30d.

In a separate experiment, the two non-lipid compartments of the multichamber bag Compleven[®], (Fresenius Kabi, Bad Homburg, Germany) which provides 5mmol Ca as Calcium Chloride (CaCl₂) and 20 mmol P (as Glycerophosphate) per 2.5L bag were combined, thoroughly mixed then transferred into five glass containers (MI, MII, MIII, MIV, MV). Lipid from the third compartment was added in the correct proportion to MIV and MV. Further Ca was added to all five samples as gluconate. P was then added to MI and MIV as FDP, to MII and MV as IP and to MIII as Addiphos[®] (Fresenius Kabi, Bad Homburg, Germany), a mixture of monobasic potassium phosphate and dibasic sodium phosphate, (IP) (equivalent to a final concentration of 20mmol Ca and 82mmol P per bag). Each combination was thoroughly mixed, inspected, then stored in the dark at 4 - 8°C and submitted to visual inspection at 1h, 24h, 4d and 30d. pH was determined at the start and finish of the experiment.

No precipitation was observed throughout the study period in any of the FDP containing regimens based on Clinomel[®], Synthamin[®], Compleven[®] or Vamin[®]. In contrast, admixture CII (containing 1.8mmol Ca and 12mmol IP per L) immediately became cloudy on addition of the extra IP and a white precipitate deposited within 1 hr.

Similarly, Compleven[®] admixtures MII and MIII without lipid, (containing 2mmol Ca and 8mmol OP per L) were unstable after addition of extra IP. An immediate, dense, white precipitate occurred in MII (initial pH 6.68) with the addition of potassium hydrogen phosphate and the pH increased initially to 7.22, and then to 7.28 over 30d storage. MIII became cloudy with the addition of Addiphos[®], (a combination of inorganic dibasic sodium phosphate and monobasic potassium phosphate) and pH shifted to 6.90. This did not change with storage and the cloudiness did not intensify.

Compleven[®] mixtures MIV and MV were designed to monitor emulsion stability of an AIO mixture. MIV remained homogenous and visibly stable throughout the storage period. MV appeared initially stable, but after 4d and increasingly through to 30d, a 'marble' effect followed by droplet coalescence developed, indicative of emulsion instability.

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Final regimens were as follows:

Regimen	SI	SII	SIII	VI	VII	VIII
Ingredients per L		Synthamin 14			Vamin 9	
Nitrogen (g)		7			4.7	
Amino Acid Total (g)		42.5			35.1	
Glucose (Kcal)		1000			1000	
Lipid (Kcal)		0			0	
IP (mmol)		2.61			0	
Addiphos (mmol)		0			0	
FDP (mmol)	4	20	40	4	20	40
GP (mmol)	0	0				
CaGluc (mmol)	2	10	20	2	10	20
Total Na (mmol)	37	45	55	27	35	45
Total K (mmol)		32.61			10	
Total Mg (mmol)		2.5			0.75	
Total Cl (mmol)		10			27.5	
Total Acetate (mmol)		70			0	
Total Ca (mmol)	2	10	20	3.25	11.25	21.25
Total P (mmol)	6.61	22.61	42.61	4	20	40

Regimen	MI	MII	MIII	MIV	MV	CI	CII	CIII	CIV
Ingredients per L			Compleven					Clinomel	
Nitrogen (g)			12.0					7.0	
Amino Acid Total (g)			30.0					42.5	
Energy Total (Kcal)			1512 + lipid					756 - lipid	
Glucose (Kcal)			360					562	
Lipid (Kcal)			400					0	
IP (mmol)	0	33	0	0	33	22	55	15	15
Addiphos (mmol)	0	0	33	0	0			0	
FDP (mmol)	33	0	0	33	0	0	0	7	40
GP (mmol)			8					0	
CaGluc (mmol)			8					8	
Total Na (mmol)	48.5	32	81.5	48.5	32	35	35	46	62.5
Total K (mmol)	20	86	20	20	86	104	170	60	60
Total Mg (mmol)			2					2.5	
Total Ca (mmol)			2					2.25	
Total Cl (mmol)			32.4					5.0	
Total Acetate (mmol)			0					112	
Total Ca (mmol)			10					12.25	
Total P (mmol)			41			22	55	22	55

Results:

Stability 0 = no visible precipitation
 + → +++ degree of visible precipitation

Adult HPN
 Clinomel®

Sample\Time	P Source	0h	1h	24h	4d	30d	pH
CI	IP	0	0	0	0	0	6.83
CII	IP	+	++	+++	+++	+++	6.83
CIII	FDP	0	0	0	0	0	6.29
CIV	FDP	0	0	0	0	0	6.29

Synthamin®

Sample\Time	P Source	0h	1h	24h	4d	30d	pH
SI	FDP	0	0	0	0	0	5.65
SII	FDP	0	0	0	0	0	5.67
SIII	FDP	0	0	0	0	0	5.65

Compleven® (without lipid)

Sample\Time	P Source	0h	1h	24h	4d	30d	pH
MI	FDP	0	0	0	0	0	6.65
MII	IP	++	+++	+++	+++	+++	7.22
MIII	Addiphos	+	+	+	+	+	6.90

Compleven® (with lipid)

Sample\Time	P Source	0h	1h	24h	4d	30d	pH
MIV	FDP	stable	stable	stable	stable	stable	6.94
MV	IP	stable	stable	stable	unstable	unstable	7.20

Paediatric HPN
 Vamin®

Sample\Time	P Source	0h	1h	24h	4d	30d	pH
VI	FDP	0	0	0	0	0	5.46
VII	FDP	0	0	0	0	0	5.63
VIII	FDP	0	0	0	0	0	5.63

DISCUSSION

Phosphate plays a vital metabolic role forming the high-energy bond within adenosine triphosphate (ATP) which fuels a wide range of physical and metabolic processes and acts as an essential regulator within the glycolytic pathway. Plasma phosphate exists in both organic and inorganic forms with a total concentration of about 3.9 mmol/L, approximately two thirds of which is organic, as a major constituent of phospholipids, enzyme cofactors and nucleic acids. The remaining third, exists as free inorganic anions but may be protein bound. The normal range for serum inorganic phosphate is 0.8-1.3 mmol/L.

Hypophosphatemia is thus defined as less than 0.8 mmol/L although neuromuscular symptoms leading to lethargy, apathy, and somnolence are unlikely until the level is lower than 0.32 mmol/L. Crohn's disease, IBS, SBS and radiation enteritis limit the absorptive capacity of the small bowel. Prolonged use of aluminium and magnesium based antacids, bind to produce insoluble complexes thus lowering bio-availability of phosphate (8). Hypomagnesaemia and hypocalcemia generally predispose to hypophosphatemia.

A chronically malnourished patient is often in a catabolic state, which is associated with muscle breakdown and subsequent loss of intracellular phosphate. Since serum phosphate remains relatively normal, this loss cannot be predicted by serum measurements. When patients subsequently receive PN they may receive insufficient P. Large volumes of carbohydrate and amino acid solutions raise endogenous phosphate requirements, and unbalanced amino acid solutions may induce further urinary P losses. If phosphate replacement is insufficient hypophosphatemia will ensue. (9) Early reports of PN-induced hypophosphatemia came mainly from USA where the energy source was exclusively glucose until the late 1980's. This problem was seen less in Europe where some phosphate has always been provided in the form of phospholipids from fat emulsions (although the bioavailability has never been clearly investigated).

Hypophosphatemia can produce symptomatic muscle weakness, poor ventricular function and poor tissue oxygenation, essentially because depletion of P leads to a decrease in high-energy substrate availability leading to respiratory muscle dysfunction. Acidosis causes breakdown of intracellular components with subsequent loss of phosphate. The kidneys are thus initially presented with high serum phosphate which is preferentially utilised as a buffer for hydrogen ions, leading to an overall phosphate deficit. (9, 10) Alkalosis, of either metabolic or respiratory origins, will produce hypophospha-

taemia, due to an intracellular shift of phosphate. (11)

An association between metabolic bone disease and PN has been known for twenty years and there are indications that between 40 and 100% of long-term PN patients have decreased bone density or histological features of bone disease (12). A recent multi-center study conducted by the ESPEN-HAN Working Group found 43% osteopenia, 41% osteoporosis, 35% bone pain, 10% bone fracture in 165 home patients across nine centres (13). PN providing insufficient amounts of Ca and P has been identified as an important risk factor.

Since Ca and P metabolism is closely linked, replacement must be carried out with regards to the complete serum Ca picture. Regular Ca and P measurements need to be taken along with serum creatine and BUN estimates. Bone mineral density correlates well with fracture risk and should be determined at the start of HPN then yearly thereafter. An increase in serum P will be accompanied by a decrease in serum Ca. Thus, with higher doses of P, simultaneous Ca administration may be indicated.

In clinical practice it is common to administer 0.4 mmol P/kg/day and to adjust this on the basis of the serum phosphate analyses. For adults on TPN, at least 10-15 mmol P and 2.5 – 15mmol Ca should be given for each 1000Kcal/d but patients at risk of osteopenia should receive at least 1500mg Ca/d (37.5mmol). Mean fractional retention rates suggest that infants and small babies may require 1.4 – 2.0 mmol Ca/Kg/d and 1.25 – 1.75 mmol P/Kg/d (14, 15). This requirement diminishes with increasing maturity and after the third year of life is around 1 mmol/kg/day. Administration of these recommended intakes can result in stable normal serum values for Ca, P, PTH, Calcitonin and Vitamin D metabolites with normal; renal tubular resorption of P (16, 17).

CaP solubility is difficult to predict and to identify. There are many contributory factors such as temperature, time, pH, type and concentration of amino acids, Ca or P additives, and formulation sequence. PN mixtures usually have a pH range of 5 – 7 at which most amino acids remain electrically neutral and exert only a weak buffer effect. This pH also optimises solubility of CaP salts. The pH of our mixtures with or without lipid ranged from 5.46 to 7.28. Whilst the use of monobasic potassium hydrogen phosphate is claimed to maintain this environment, raising pH increases the proportion of dibasic phosphates and the probability of Ca salt precipitation, as borne out by our results. Addition of IP consistently produced higher pH values, with a greater likelihood of precipitation than addition of FDP. Moreover, addition of Addiphos® (a mixture of inorganic phosphates) to Compleven® produced a lower pH 6.90 compared to that obtained from addition of the dibasic IP alone (MII, pH 7.22). As a result, the stability of the CaP com-

plex was greatly reduced in the latter mixture observed as a dense, white precipitate in contrast to the cloudiness of admixture MIII. A recent UK survey (18) reports that approximately 50% of compounding units incorporate CaCl_2 rather than Calcium Gluconate (CaGluc) into HPN admixtures. In contrast, CaCl_2 is not recommended in the USA for PN because it is more dissociated than Calcium Gluconate (CaGluc). (19) However, body temperature (37°C) may increase the dissociation of CaGluc and hence the risk of interaction between free Ca and dibasic inorganic HPO_4^{2-} ions leading to precipitation. (20) We nevertheless standardised with CaGluc for our studies at RT and refrigeration temperature.

The probability of CaP precipitation is further heightened when additives are incorporated into all-in-one (AIO) lipid-containing admixtures. These increasingly complex regimens, containing up to fifty different nutrients will create a proportional increase in chemical interactions, often with opposing effects on stability. Two predominant factors can effect AIO stability. One is the presence of electrolytes, such as Ca, which may induce coalescence of the emulsion particles. The second factor is pH, which may cause separation of the emulsion. Both factors effect the relative ionic strength of the mixtures and can change the negative potential at the surface of the emulsion particles, which normally maintains globule separation. (4) High strength dextrose for example, increases mixture viscosity, which improves CaP solubility by restricting ionic mobility, but also lowers pH which can adversely impact on emulsion stability. Dextrose may also increase calciuresis (21). Whether or not lipid contributes to CaP stability or vice versa is complex and still debatable. One argument suggests that Ca binding to the phospholipid emulsifier limits the chance of Ca and P interaction thus reducing the likelihood of precipitation. We believe it is more likely however that 'bridging' between the Ca cations and the negatively charged phospholipid groups of the lecithin molecules induce lipid aggregation and hence instability of the emulsion. On the other hand, lipid addition in the presence of inorganic salts has been reported to cause an increase in mixture pH, which favours CaP precipitation. To some extent this was our experience. The less stable mixture MV containing IP, consistently recorded a pH approximately 0.3 higher than the stable mixture MIV containing FDP. This would support the concept that an excess of cations (i.e. Ca) in the mixture MV reduce the zeta potential and electrophoretic mobility of the emulsion particles, leading not only to CaP precipitation, but also to admixture instability. In contrast, by chelating with the Ca, FDP has an opposite effect leading to improved stability. (5)

Ideally, all nutrients should be assessed for AIO ad-

mixture stability but this is not currently practicable. Most laboratories concentrate on detecting and measuring stability markers such as precipitates, oxidation products or the physical characteristics of emulsions. Particle size distribution testing (PSD) of the emulsion mixtures would offer further confirmation of our findings. However, based on experience in our laboratories with many AIO admixtures over almost 20 years, the PSD technique was not deemed necessary in this study due to the dramatic and obvious instability created when extra Ca and IP were added to these admixtures.

Organic phosphates such as FDP offer a more practical and physiological solution that has been pursued with considerable success in Italy and other European countries for almost 20 years. They consist of a P group covalently bonded to an organic molecule such as glycerol, glucose or fructose. The P group is not fully ionised and therefore much less available for adverse interaction with calcium. As natural substrates for extracellular phosphatases, they are well tolerated with a lack of significant toxicity, even if dosage exceeds 10 times normal daily parenteral P requirements.

Fructose-1,6-diphosphate (FDP) is a metabolic intermediate of glycolysis and is utilised by cells for the production of ATP without energy consumption. This is achievable due to the bypassing of the critical ATP requiring step catalysed by phosphofructokinase. FDP appears to enhance Ca cellular uptake in the early stages of ischemia (22) and inhibits proliferation and migration of endothelial/smooth muscle cells (23).

A balance study in very low birth weight (VLBW) babies, which used FDP enabled optimum Ca and P ratios (1:1.3) to be administered: 54mg(1.35mmol)/Kg/day Ca, 54mg(1.75mmol)/Kg/day P, demonstrating high retention rates (40-60% for Ca, 80-90% for P) with no precipitation problems encountered (24). Markov et al have recently looked at metabolic responses to FDP infusion in healthy adults (25). Results indicated a significant increase in serum inorganic phosphate and intraerythrocytic ATP levels. Intravenous FDP has been generally advocated in the treatment of acute or chronic hypophosphataemic conditions, (26) and was found to be beneficial by increasing respiratory muscle strength in malnourished chronic obstructive pulmonary disease (COPD) patients (27).

Ca:P ratios may be important to achieve optimal mineral retention, especially in pediatric HPN. Ratios <1:1 have a potential for disturbing Ca and P homeostasis. In the UK, clinical ratios are preferred to be closer to 1:2 (18) but this has been difficult to achieve in pharmaceutical practice and few balance studies have been reported. A major advantage of FDP, confirmed by our results, is the fact that P can be provided in twice the molar ratio

compared to other inorganic or organic P sources. This could be vital in fluid volume and/or sodium restriction (28). It also increases the diversity and scope for specifically tailored HPN regimens that meet the clinical optimum ratio of 1:2 and are otherwise limited with compounds providing Ca and P in a fixed 1:1 ratio.

CONCLUSION

Wider use of organic phosphates was advocated in 1996 (29) on the basis of physicochemical, biochemical, pharmacological, pharmaceutical and clinical hypotheses.

Our results confirm that high concentrations of Ca and P precipitate, when supplementary IP is added to 'standard' Ca/P containing HPN regimens, but not when added as FDP. By using suitable combinations of FDP (Esafosfina®) with calcium gluconate, it is possible to prepare a variety of stable HPN admixtures, containing up to 20 mmol Ca with 82 mmol P per 2.5 – 3L adult

TPN bag (20mmolCa:40mmolP/L for paediatrics), sufficient to meet most clinical requirements. Moreover, by providing as much Ca and P as clinically required, in the more appropriate ratio of 1:2 or greater, PN associated metabolic bone disease may become less of a debilitating problem for home patients. The longer shelf lives of up to 30 days for those stable FDP containing admixtures that we tested should further ensure a safe and convenient supply chain of product to HPN patients.

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