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The destabilization of parenteral feeding emulsions by heparin

O.L. Johnson [1], C. Washington [1], S.S. Davis [1] and K. Schaupp [2]

[1] *Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham (U.K.) and* [2] *Leopold & Co., Graz (Austria)*

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Summary

We have investigated the destabilization of fat emulsions (Intralipid 20%) in TPN mixtures containing heparin. Measurement of the droplet charge (ζ-potential) indicated that heparin flocculates emulsions when the droplet charge is low or charge-reversed in the presence of divalent cations. Since these occur in the vast majority of total parenteral nutrition mixtures, the addition of heparin to such mixtures is strongly contraindicated.

Introduction

Total parenteral nutrition (TPN) mixtures contain a fat emulsion, glucose, amino acids and electrolytes to make up the nutritional requirements of the patient. Although the unmixed fat emulsions are stable for several years, TPN mixtures can be unstable due to emulsion flocculation and coalescence (see e.g. Sayeed et al., 1987). They are normally used within 48 h of mixing. The instability arises because the charge on the emulsion droplets, which stabilizes the emulsion by mutual droplet repulsion, is decreased in the presence of electrolytes. This phenomenon has been widely studied, and is well understood in simple emulsion–electrolyte systems containing no other component (see e.g. Davis, 1982).

Recently there have been reports on the incompatibility of TPN mixtures and heparin (Rauff et al., 1988; Rattenbury et al., 1988). The incompatibility results in the production of emulsion aggregates or flocculates in the presence of heparin. The reports also suggest that the flocculation is the result of interactions between calcium ions, the fat emulsion and heparin, since flocculation only occurred when both calcium and heparin were present. The most striking feature of the reports is the speed with which the system flocculates, separation of a cream layer being visible within 1–2 minutes.

The droplets of fat emulsions stabilised by the phospholipid material lecithin have a net negative charge of -30 to -50 mV. The major phospholipid in lecithin is phosphatidylcholine, which is uncharged at physiological pH. The negative charge is imparted by the presence of phospholipids which are negatively charged at pH 7.0 (Davis et al., 1982). These include phosphatidylserine, phosphatidylglycerol and phosphatidic acid, which

Correspondence: C. Washington, Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.

make up 1–2% of the mass of the refined lecithins used in the production of fat emulsions.

Addition of charged species such as electrolytes and amino acids (depending on the pH), affects the surface charge and consequently the stability of the fat emulsion. We can distinguish two fundamental types of surface interaction with electrolytes. Non-specific adsorption occurs when the ions adsorb to the surface due to the attractive surface potential. Monovalent cations such as sodium and potassium behave in this manner on lecithin surfaces. Specific adsorption occurs when the cation is chemically complexed or bound by groups on the droplet surface; divalent cations such as calcium and magnesium fall into this group with respect to lecithin-stabilized fat emulsions. Whereas non-specifically adsorbed ions can only neutralize a surface charge, specifically adsorbed ions can cause charge reversal. Thus sodium would neutralize the negative charge on a fat emulsion, but sufficient calcium would cause a positive charge to develop (which could even restabilize the emulsion at high calcium concentrations).

We have measured the surface charge of 'Intralipid 20%' in the presence of calcium, and the corresponding flocculation behaviour in the presence of heparin, in an attempt to understand the nature of the flocculation.

Materials and Methods

Heparin sodium 5000 IU/ml (Multiparin, batch 8610527 2) and Intralipid 20% (Kabivitrum, batch 69643) were obtained from the hospital pharmacy, Queen's Medical Centre, Nottingham. Calcium chloride and sodium chloride were B.D.H. Analar grade, and were used as received.

Mixtures of Intralipid 20% (10 ml) and calcium or sodium chloride solution (10 ml) containing heparin (5 I.U./ml) were prepared and their flocculation was observed visually. The final electrolyte concentrations were: calcium chloride 0–20 mM, sodium chloride 0–500 mM. One hour after mixing, the particle size of the aggregates was determined by dilution into distilled water followed by size analysis (2600 diffraction sizer, Malvern Instruments, U.K.).

Surface (ζ) potentials at 25 °C were measured using a Zetasizer II (Malvern Instruments, UK.) fitted with a PC3 narrow-bore cell. 'Intralipid 20%' was diluted into the appropriate concentration of electrolyte before measurement.

Results

Table 1 shows the ζ-potential and particle size data of Intralipid 20% in the presence of calcium and heparin. Intralipid 20% had a ζ-potential of −38.2 mV in the absence of calcium. This was rapidly neutralized as calcium was added, the point of zero charge occurring between 2 and 5 mM. Flocculation was observed visually for all mixtures containing calcium, and this is confirmed by the particle size analysis.

Table 1 shows the ζ-potential and particle size data of 'Intralipid 20%' in the presence of sodium and heparin. The charge was slowly neutralized as sodium was added, but a point of zero charge was not reached in the concentration range studied (0–500 mM). Flocculation was not detected visu-

TABLE 1

Effect of electrolytes on ζ-potential and heparin-induced flocculation of Intralipid 20%

Conc. (mM)	$d_{90\%}$ (μm) * ($n=4$)	ζ-Potential (mV) ($n=5$)	Flocculation
Calcium			
0	1.28±0.02	−38.2±0.1	−
1	5.54±0.08	−4.5±0.1	+
2	8.65±0.48	−3.5±0.2	+
5	9.26±0.85	+1.6±0.4	+
10	6.60±0.11	+4.6±0.3	+
20	7.50±0.37	+4.1±0.5	+
Sodium			
0	1.20±0.02	−35.5±1.2	−
10	1.21±0.02	−30.5±1.0	−
50	1.21±0.02	−17.5±5.0	−
100	1.22±0.02	−10.1±1.0	−
500	1.26±0.02	−15.1±1.0	−

* 90% of the droplets had a diameter less than the value mentioned.

ally or by particle size analysis in any of the mixtures containing sodium and heparin.

The particle sizes quoted are $d_{90\%}$, i.e. 90% of the particles were below the given size. It should be noted that the 2600 diffraction sizer used in these experiments is insensitive to droplets below 1.2 μm. Consequently the sizes are biased in favour of the larger droplets. This gives a good characterization of the particle size distribution above 1 μm, but the numerical values are larger than those which would have been obtained if the entire particle size distribution had been sampled.

Discussion

The zeta potential measurements indicated that a low concentration of calcium ions (1 mM) brought about a large reduction in the stabilizing ζ-potential (from -38 mV to -4 mV) on the Intralipid droplets. A further increase in the concentration of calcium ions resulted in the reversal of the surface charge. This is in agreement with data reported previously. Notwithstanding the reduction in charge, Intralipid 20% would normally be expected to flocculate only slowly (over several days) in the presence of calcium of the concentration range used here.

When the charge-reversed emulsion was mixed with heparin, a negatively charged polyelectrolyte, the flocculation rate increased dramatically, leading to visually observable separation within minutes. We propose that this is due to heparin acting as a bridging flocculant between oil droplets; that is, when the oil droplet charge falls below approximately -5 mV, heparin can interact with the positively charged sites on the droplet surface caused by calcium binding.

It is probable that a positively charged site on the surface results from calcium binding even at low calcium concentrations, when the number of such sites is low, and the overall droplet charge is still negative. However, heparin would be unable to interact with such sites since the overall negative droplet charge would produce a predominantly positive ionic solution layer, depleted in negative species (such as heparin). Only when the droplet charge falls below a critical value can the heparin complex interact with the positive surface site. This is supported by the observation that sodium and heparin together do not flocculate emulsions, since the highest sodium concentration studied (0.5 M) only reduced the droplet charge to -15.1 mV. At this concentration there was presumably a sufficiently positive ionic solution layer to prevent the droplet interaction with heparin. The model that results is one of an irregular pattern of positive and negative charges on the droplet surface, shielded from the environment by a uniform ionic double layer whose thickness and sign depends on the overall droplet charge.

Since heparin interacts with neutral or positive droplets, its effect is to accelerate flocculation which would occur slowly in the presence of calcium alone. This acceleration effect is considerable since separation is observed in minutes instead of hours. At high calcium concentrations fat emulsion becomes positively charged; in this region, the presence of heparin causes rapid flocculation of the positively charged droplets.

Conclusions

The results suggest that the flocculation of TPN mixtures in the presence of heparin is a consequence of the calcium ion content. Furthermore, the flocculation depends on the ability of divalent cations and presumably other polyvalent cations to reverse the surface charge of the emulsion droplets. It is likely that replacement of calcium by magnesium would also lead to similar results to those reported here. Such positively charged emulsion droplets are flocculated by bridging heparin molecules, leading to rapid flocculation of the emulsion.

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