J. Pharm. Pharmacol. 1995, 47: 566–570
Received July 22, 1994
Accepted January 10, 1995

The Effect of Lysine, a Water-structure Breaker, on the Stability of Phospholipid-stabilized Emulsions

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Abstract

Phospholipid-stabilized emulsion properties were studied in the presence of lysine, a water-structure breaker, using two unrelated procedures, photon correlation spectroscopy and a light obscuration instrument. Commercial Intralipid was used as a control.

Lysine 0·125, 0·25 and 0·5 M induced changes in the size distribution of a non-heated model emulsion system, irrespective of any changes produced by environmental pH. Some of the laboratory-prepared emulsions containing lysine were more stable than the corresponding commercial heat-sterilized product Intralipid, once heated.

The results suggest that lysine is producing an effect on the nascent oil–water interface that controls the physical stability of the system. Once the heat-induced interfacial rearrangement of the individual phospholipid molecules occurs, the influence of lysine becomes diminished.

First introduced into clinical practice for parenteral nutrition over thirty years ago, phospholipid-stabilized emulsions such as Intralipid (Pharmacia, Stockholm, Sweden) have been extensively studied and, in general, shown to possess remarkable physical stability. This has led to the development of extemporaneously prepared total parenteral admixtures in which amino acid and dextrose solutions are mixed before administration to the patient, the mixtures often containing materials such as trace elements and vitamins, total parenteral nutrition (TPN). For the most part, these systems have remained physically satisfactory from a stability point of view but occasional reports of the emulsion destabilization have appeared (P. Wright, Coordinator, National TPN Group, UK, personal communication) although the exact mechanism for these reactions remains at present somewhat obscure (Lutz et al 1994).

Since there had been earlier reports that amino acids were associated with water-structure breaking activity (Kay & Evans 1965, 1966; Kay 1968), Lutz et al (1994) carried out a systematic evaluation of the effect of amino acids on water. All 21 amino acids tested had some breaking activity on bulk water structure but three, lysine, glutamic acid and aspartic acid, and their respective salts, had pronounced water-structure-breaking activity. On a molar equivalent basis this could be reversed by addition of dextrose, a known water-structure maker. This observation suggested that the order of addition of total parenteral admixtures may have a profound effect on bulk water structure which, conceivably at least, might have a parallel effect on the vicinal water of the oil/water interface, leading to destabilization of the emulsion system. Thus, the dextrose should be added to the emulsion first, followed by the amino acid solution in order to optimize the stability of the TPN admixture.

Groves & Herman (1993), on the other hand, demonstrated that the phospholipid composition of the oil and the water phases of freshly prepared emulsions, broadly similar in constitution to Intralipid, changed after the application of heat. This was interpreted as being due to the heat-induced formation of a stable mesophase at the oil/water interface that stabilized the system from the type of coalescence reaction associated with unstable emulsions (Kitchener & Mussellwhite 1968). Since Intralipid is terminally heat-sterilized, this may in itself account for the remarkable stability of this oil-in-water emulsion to thermal challenges and, for the most part, to the addition of other components of TPN admixtures.

Nevertheless, this present investigation was initiated to determine if the changes previously detected in bulk water structure could be translated to changes being detected in the stability profile of a phospholipid-stabilized emulsion following the addition of lysine or its salts. We have evaluated the effect of adding the amino acid to freshly prepared emulsions with coarse and fine particle-size distributions, both before and after heating, using commercial Intralipid as a control, since this was both finely divided and heat sterilized.

Materials and Methods

Materials

L-Lysine base, lysine hydrochloride (both >99% purity), were obtained from Fluka Chemical, Ronkokoma, NY. Injectable grade egg phospholipids were from Pharmacia Inc., Clayton, NC (Lot #K1317). Soy oil was from Jewel Grocery Company Inc., Chicago, IL, Intralipid (20%), Lot #58314A was from Pharmacia Inc., Clayton, NC. Glycerol and other chemicals were from Fisher Scientific, Itasca, IL.

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Methods

Preparation of emulsions. Glycerol (2·25 g) was dispersed in water by stirring followed by egg phospholipids (1·20 g), and stirring continued until the solution was optically clear. When appropriate, lysine, lysine hydrochloride or a mixture of the two was added at this stage, to provide a final lysine concentration of 0·25 M. Soy oil (20 g) was added and the system made up to volume 100 mL with purified water and the first, coarse, emulsion prepared by dispersion with an Ultra Turrax homogenizer (Model TP18, 10SI Janke & Kunkel AG, Ika Werk, Germany) at 20 000 rev min^{-1} for 12 min. This provided a coarse emulsion that was stable under ambient conditions although the oil phase could be separated by prolonged centrifugation. A finer emulsion that could not be separated centrifugally was prepared by homogenizing the same system through a Microfluidizer (Model 110Y, Microfluidics, Newton, MA) at a liquid pressure of 10 000 psi. The pH of the final emulsions was unadjusted (Table 1) or adjusted to pH 7·3 with 0·01 M sodium hydroxide. Completed emulsions were packed aseptically in butyl-rubber sealed 3-mL glass vials, under nitrogen, and were stored under refrigerator conditions, ~ 5°C, or as described.

pH measurement

The pH of all emulsions was measured at ambient room temperature (21°C) after adding potassium chloride (100 mM final concentration) using a calibrated glass electrode instrument (Model SA210 Orion, Boston, MA).

Centrifugal stress

Emulsions were stressed by centrifuging 35-mL samples in 50-mL polypropylene calibrated centrifuge tubes (Gibco, Gaithersburg, MD) using an IEG/S11 centrifuge Damon/IEC Division, Needham, MA) at 1500 rev min^{-1} for 90 min at a temperature of 40°C. The volume of free oil was measured where possible but none was observed in Intralipid 20% or in the homogenized emulsions under these conditions.

Freeze-thaw cycles

Emulsions were stressed by systematic freeze-thaw exposure, cycling vials for 24 h at 30°C followed by another 24 h at −20°C. The total content of each vial was taken for analysis at the end of the 30°C phase of the cycle.

Particle-size measurements

The mean particle size of the emulsion systems was determined by photon correlation spectroscopy (PCS) using a Nicomp Model 270, version 3·0 Particle Sizer (HIAC/Royco Instruments Division, Silver Springs, MD) after 1:1000 dilution with purified water. Particles larger than 2·0 μm were counted after dilution to 2000 with purified water using a HIAC/Royco Model 3000 sampler fitted with a HRLD-150 laser sensor coupled to a Model 4100 particle counter (HIAC/Royco Instruments Division, Silver Springs, MD).

Admixtures

Model total parenteral admixtures were prepared under aseptic conditions. Lysine base, lysine hydrochloride or a (0·7:99·3) mixture of the two was dissolved in 2·5% aqueous glycerol and diluted 1:1 with Intralipid 20%, effectively preparing the equivalent of Intralipid 10% but with a pH range of 5·6 to 9·7. Final lysine base concentrations of 0·125, 0·25 and 0·5 M were prepared, with a control consisting of Intralipid 20% diluted with an equal volume of 2·5% aqueous glycerol. Mixtures were stored in 3-mL butyl-rubber stoppered glass vials under refrigerator conditions (~ 5°C) until required for evaluation and testing.

Results

Measurements of the mean droplet size by PCS for the different emulsion systems are summarized in Table 2. Maintaining the pH of the emulsions at 7·3 by addition of sodium hydroxide after the addition of lysine base showed that the mean diameter of the homogenized system increased in a linear fashion as the lysine was added (Fig. 1). Since this system was stable to centrifugal stress, the experiment was repeated using the coarser emulsion. Again, systematic addition of lysine produced a direct effect seen as the oil separated out after centrifugal stress (Fig. 2).

Few particles larger than the 2·0 μm size threshold were evident in Intralipid or in the control homogenized system. When the latter system was prepared with increasing

Table 1. The mean number of droplets > 2 μm diameter for Intralipid emulsion systems (equivalent to 10% Intralipid) exposed to up to 19 freeze-thaw cycles (−20/30°C after admixture with lysine.

Emulsion formulation	Lysine concn (M)	pH	Mean number of droplets > 2 μm, after 19 freeze-thaw cycles (×10^7 mL^{-1}, related to the undiluted Intralipid 20%)[a]
Control	0	8·10	5·181 ± 0·168
Lysine base	0·125	9·97	5·341 ± 0·549
	0·25	10·01	5·231 ± 0·337
	0·50	10·05	4·972 ± 0·283
Lysine hydrochloride	0·125	6·56	5·576 ± 0·857
	0·25	6·35	5·360 ± 0·518
	0·50	6·15	5·657 ± 0·569
Combination	0·125	7·21	5·156 ± 0·141
	0·25	7·19	5·199 ± 0·406
	0·50	7·18	5·000 ± 0·387

[a] The values for the number of droplets represent the mean ± s.d. over the time scale of the experiment. n = 19 for each lysine concentration except for the control where n = 9.

Table 2. The mean droplet size measured by photon correlation spectroscopy (PCS) for the three emulsion systems studied.

Emulsion 20%	PCS diameter (nm)
Intralipid	354 ± 53
Coarse emulsion	680 ± 413
Homogenized system	258 ± 21

Mean ± s.d.

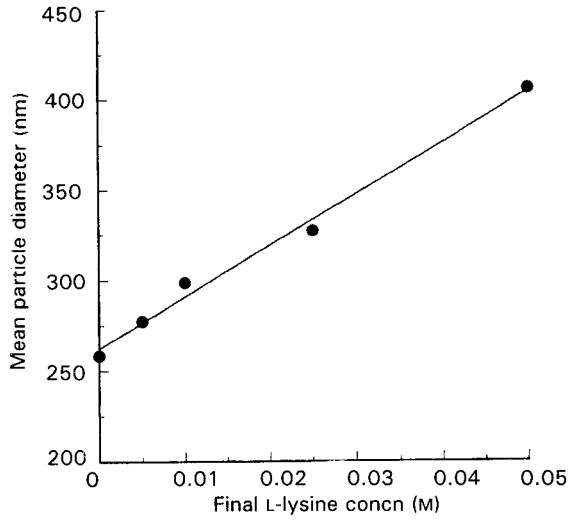

FIG. 1. The effect of lysine on homogenized non-heat stressed 20% o/w emulsion at pH 7·3.

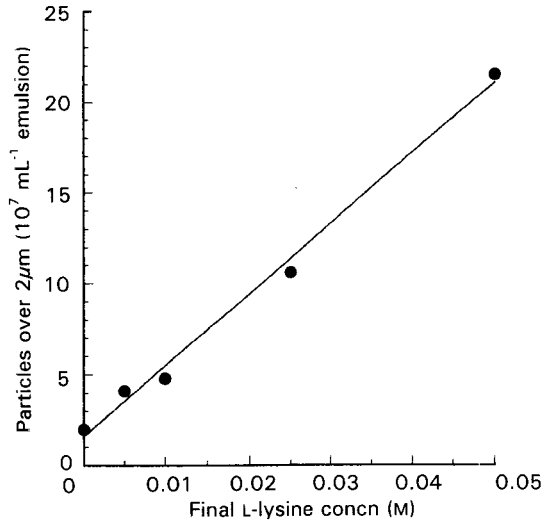

FIG. 3. The effect of lysine on the number per mL of droplets >2·0 μm diameter for homogenized non-heat stressed emulsions.

amounts of lysine, the number of particles above this arbitrary size threshold steadily increased (Fig. 3). These numbers did not change over a period of 14 days storage at ~ 5°C (Fig. 4).

The data strongly suggest that the presence of lysine in these non-thermally stressed emulsion systems produced a profound change in the state of the oil dispersion. This must be due to some influence at the oil–water interface, which in turn affects the initial coalescence process at the moment of emulsion interface formation.

The effect of heat on the coarse freshly prepared emulsion was evaluated after heat exposure at 95°C for 30 min since these conditions had been demonstrated by Groves & Herman (1993) to produce the irreversible exchange of phospholipids that suggested formation of a stable mesophase. Results are summarized in Table 3. Statistical analysis (Student's *t*-test, significance level of 5%) suggested that there was a direct effect due to pH on diluted Intralipid 20% systems but, superimposed on this observation, there was also an affect attributable to lysine (Table 1). This may also be seen intuitively in Table 3 since, at pH 7·3 (the admixture of lysine base and hydrochloride), there is a marked destabilization effect on the coarse emulsion compared with the system at pH 6·3, without lysine.

The effect of freeze-thaw cycling on the size of the model admixture as measured by light obscuration using, effectively, dilutions of Intralipid 20% with lysine base, hydrochloride or a combination of the two, is shown in Table 4. Even after 19 cycles there was no gross separation and little or no significant effect on the physical stability could be

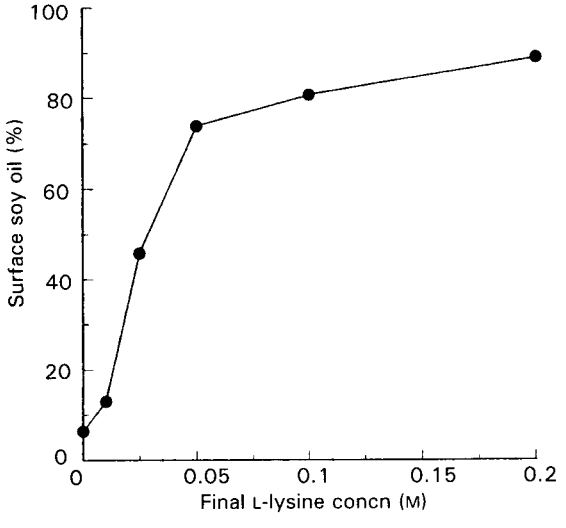

FIG. 2. The effect of lysine on the stability of non-heat stressed coarse emulsions expressed as the percentage volume of free interfacial oil following centrifugation.

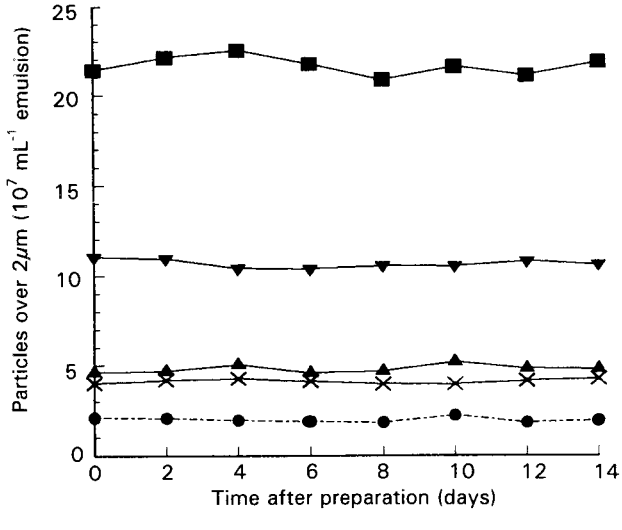

FIG. 4. The particle count of droplets >2·0 μm following the addition of various concentrations of lysine to homogenized non-heat–stressed emulsions over 14 days storage at ~ 5°C. ● Control, L-lysine, X 0·005 M, ▲ 0·01 M, ▼ 0·025 M and ■ 0·05 M.

Table 3. The effects of heating, storage or adding lysine on the separation of free oil following centrifugal stress on a coarse emulsion at different pH values.

pH of 0·25 M L-lysine admixed 20 (o/w) emulsions	Soy oil (%) at the surface after centrifugation		
	Emulsions		
	Immediately after preparation	Heat-stressed (95°C 30 min)	Non heat-stressed (approx. 5°C 3 days)
5·6	82	89	
6·3 (control, 0 lysine)	12	12	5
7·3	75	76	10
9·7	52	40	18

determined. Fluctuations that were seen are readily attributable to the experimental and sampling errors inevitable in this type of procedure. However, Intralipid itself showed some coalescence after eight cycles.

Discussion

The measurement of particle size and size distribution in an injectable emulsion system requires discussion since it remains technically difficult. A number of authors have established that the mean size of a homogenized phospholipid-stabilized oil-in-water emulsion, typified by the Intralipid products, is generally between 200 and 400 nm, depending on the volume of the oil phase (Lee & Groves 1981; Takamura et al 1984; Washington 1992). However, to extend a particle size distribution analysis into the micron-sized region is difficult, even with modern instrumental technologies. The nature of a size distribution curve would imply that numbers (per unit volume) of particles above 1 μm in diameter could be found but measuring these, and relating the numbers to the mean size obtained by suitably sensitive methods such as photon correlation spectroscopy, becomes problematic. Moreover, when considering the physical degradation mechanisms of emulsions that produce changes in the size distribution, as the droplets grow larger by coalescence, it is the larger particles in the system that will provide information about these changes, albeit incompletely (Li et al 1993). Accordingly we elected to measure emulsion properties by two unrelated procedures, utilizing PCS and a light obscuration instrument. This latter instrumental principle has been shown to be sensitive enough to measure small numbers of subvisual particles present in injection solutions (Groves 1993). Because of the presence of large numbers of sub-instrumental threshold particles present in the diluted emulsion as it passes through the sensor, there may be some non-linearity of the instrument response although this effect may not be severe (Groves 1993). For comparative purposes, however, we suggest that the procedure is likely to detect physical changes that ultimately produce large particles in the various systems as a function of the applied stress.

This present investigation demonstrates that lysine, a water-structure breaker, is capable of inducing changes in the size distribution of a non-heated model emulsion system, irrespective of changes produced by environmental pH. These changes include the amounts of oil centrifuged out of the dispersed state as well as changes in the >2 μm

Table 4. The particle count of droplets >2 μm diameter for the control Intralipid emulsion system exposed to up to 19 freeze-thaw cycles (−20/30°C).

Number of freeze-thaw cycles	Number of droplets (10^6 mL^{-1} undiluted Intralipid 20%)			
	>2 μm	>5 μm	>10 μm	>15 μm
0	52·716	2·770	0·038	0·008
1	53·188	3·353	0·093	0·018
2	51·684	3·369	0·114	0·020
3	50·203	3·186	0·166	0·038
4	50·886	4·369	0·221	0·034
5	51·098	5·454	0·228	0·046
6	52·795	4·043	0·134	0·023
7	52·916	3·868	0·200	0·026
8	54·350	5·961	0·615	0·132
9	76·169	3·874	0·244	0·060
10	115·148	6·152	0·284	0·058
11	110·110	7·404	0·205	0·033
12	122·174	8·038	0·359	0·071
13	101·750	6·093	0·255	0·061
14	161·765	18·985	0·965	0·114
15	160·000	16·421	0·660	0·130
16	141·879	12·019	0·629	0·134
17	213·390	30·442	1·283	0·142
18	186·558	18·841	0·943	0·190
19	213·343	41·158	3·451	0·835

particle numbers. These changes imply that the lysine is producing an effect on the nascent oil–water interface that ultimately controls the physical stability of the system. Once the heat-induced interfacial rearrangement of the individual phospholipid molecules occurs, presumably forming a stable mesophasic system, the influence of lysine becomes diminished. For the purpose of the present discussion, it is not necessary to define precisely what effect the lysine is exerting, merely that an effect can be detected.

It should be noted that the homogenized model phospholipid-stabilized emulsion is relatively stable once it is heated and the corresponding commercial heat-sterilized product Intralipid is also relatively stable (Table 3). However, some of the laboratory-prepared emulsions containing lysine are more stable than Intralipid which again suggests interfacial involvement. Nevertheless, as discussed by Idson (1988), even resistance to eight freeze-thaw cycles is confirmation of the remarkable stability of the Intralipid emulsion. This is confirmed by the way the numbers changed on a further 11 freeze-thaw cycles (Table 1).

As emphasized by Lutz et al (1994), the order of mixing for a total parenteral admixture may be critical, it being suggested that amino acid mixtures should be added to the system last. In part, this is to minimize any possible effects due to water-structure breaking, but also to allow water-structure makers such as dextrose in the system to overcome any untoward effects that might be produced by the amino acids.

Acknowledgements

We wish to acknowledge with gratitude the support for this project provided by Dr Roland Jeppsson and Pharmacia Inc., Clayton, NC.

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