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A comparison of the relative sequestration of free and albumin-bound phenytoin by Intralipid: further insights to determine the utility of the Lipid Sink in clinical practice

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A comparison of the relative sequestration of free and albumin-bound phenytoin by lipid emulsion: further insights to determine the utility of the Lipid Sink in clinical practice

Intravenous lipid emulsion (ILE) continues to generate interest as a treatment for lipophilic drug toxicity. The ability of ILE to create an intravascular lipid compartment with the capacity to sequester lipophilic drugs is described as the 'lipid sink'.

Drug carriage within the intravascular compartment involves several distinct physiological elements, namely the aqueous phase, red cell transport and protein carriage (including alpha-1-acid glycoprotein and albumin). Of these, the protein compartment and specifically albumin is deemed to be most important and many lipophilic drugs are known to be highly protein bound. With this in mind, the potential for drug binding by ILE may be influenced by the presence of albumin. Recent work on amitriptyline, (which is 95% albumin bound) demonstrated lipid modified into nanospheres could sequester 60% of the drug when it was present in an aqueous medium but only 24% when presented in an albumin solution [1]. Moreover, the relative percentage of amitriptyline sequestered by ILE increases as the concentration of amitriptyline increases [2], an effect also observed with other drugs [3]. I undertook to study the ability of Intralipid to sequester phenytoin in both albumin and an aqueous albumin-free solution. The hypothesis for this study was that Intralipid would sequester phenytoin to a significant extent in all solutions, but to a greater extent when a higher free concentration of phenytoin was present.

Methods

After consultation, the National Research Ethics Service advised the protocol for this laboratory study exempted it from the requirement to obtain formal ethical approval. Phenytoin (Hospira, Leamington Spa) diluted in Hartmann's solution (Baxter, Thetford) was prepared at initial concentrations of 833 $\mu mol.l^{-1}$ and 83 $\mu mol.l^{-1}$. Similarly phenytoin solutions of identical concentrations in 4.2% albumin were also prepared. These solutions were calculated to provide a

final albumin concentration of 33 $g.l^{-1}$ once the dilutional effect of the additional solutions associated with the study had been taken into account.

Then 28.5 ml of each solution were used as follows: One third of the original volume (9.5ml) was diluted with 2.5 ml Hartmann's solution, and divided in to two 6ml controls. Three millilitres of 20% Intralipid (Fresenius Kabi, Runcorn) was added to the remaining 19 ml and mixed for 20 min, emulating ILE therapy approximating to 500ml Intralipid in a 75 kg subject. Thereafter this sample was equally divided into two test tubes, each containing 11ml. Then 25000iu (1ml) heparin (Wockhardt, Wrexham) was added to each test tube and mixed to flocculate the lipid [4]. The pH of all samples was monitored. Following separation overnight, the aqueous subnatant of each sample was drained with phenytoin and lipid concentrations and protein content measured using spectrophotometry and electrophoresis respectively. Similarly phenytoin concentrations were measured in each control in order to calculate the extent of sequestration of phenytoin by Intralipid. The study protocol was repeated five times yielding ten results for each solution tested.

Results

All sample pH values were between 7.04 – 7.34. Protein electrophoresis, coagulation tests and total protein values indicated no protein was removed using the experimental methodology employed. Conversely, lipid separation following heparin flocculation was virtually complete. Measured and calculated phenytoin sequestration results are summarised in Table 1. Notably, the degree of phenytoin sequestration in the high phenytoin-concentration albumin solution mirrors that observed in the low phenytoin-concentration Hartmann's solution (Figure1).

Discussion

The lipophilic drug phenytoin was selected because it is 90% protein bound at the concentrations used in this study, it is unionised at physiological pH (Log P = Log D(7.4) = 2.52), and easily assayed. The phenytoin concentrations used in this study extend from therapeutic (low phenytoin-concentration samples) to toxic (high phenytoin-concentration samples) levels. The magnitude of the reduction of the free unbound phenytoin is substantial. This confirms the

presence of the “lipid sink” and implies ILE could have a role in treatment of phenytoin overdoses. Hence, the results are of direct clinical importance.

This study demonstrates that the percentage of sequestration is related to the initial free drug concentration (Figure 2). This is particularly relevant to drug overdoses especially if carrier protein drug binding capacity in the plasma has been exceeded. It indicates that the capacity of ILE to sequester phenytoin is huge, although the actual percentage sequestered is dependent on the free aqueous phenytoin concentration. Interestingly, the extent to which phenytoin sequestration by ILE occurs in the high phenytoin-concentration (630.3umol.l^{-1}) albumin solution (in which phenytoin is 90% bound) is of a similar magnitude to the sequestration of phenytoin in the low phenytoin-concentration (61.4 μmol.l^{-1}) Hartmann’s solution. In both situations the final concentration of *free* phenytoin is very similar (37.0 vs. 33.3 μmol.l^{-1}) (Table 1).

As Intralipid sequesters free drug it is probable that this free proportion is replenished from the albumin stores, signifying Intralipid has the capacity to bind more drug. This implies that equilibrium between the drug in the lipid and aqueous compartment does not necessarily maintain a set ratio. At extremely high concentrations this process does not hold, possibly because of lipid sink saturation. Theoretically, phenytoin should have a free/lipid ratio of 1:Antilog $P_{phenytoin}$ (i.e. 1:316) at all concentrations, which does not seem to be the case (Table 1). This study shows that previous work using aqueous solutions can be replicated in Albumin containing solutions and adds to our understanding of the interactions between study drugs and physiological media.

The relatively ineffectual sequestration of phenytoin by ILE at low concentrations (approximating to the clinical therapeutic range) appears to be a fortuitous, but welcome, finding. This implies that phenytoin might be used, concurrently with Intralipid, to control convulsions manifesting from overdose of an unrelated drug as illustrated in a recent case report [6].

Conclusion

This study supports the potential benefit of ILE in the treatment of phenytoin overdose. As the concentration of free drug increases sequestration by the lipid sink appears more effective and implies drug removal occurs predominantly from the aqueous compartment. It is probable that the exact mechanism by which the ILE sequesters drugs present at toxic concentrations appears to be more complex than at first thought.

References

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Table 1

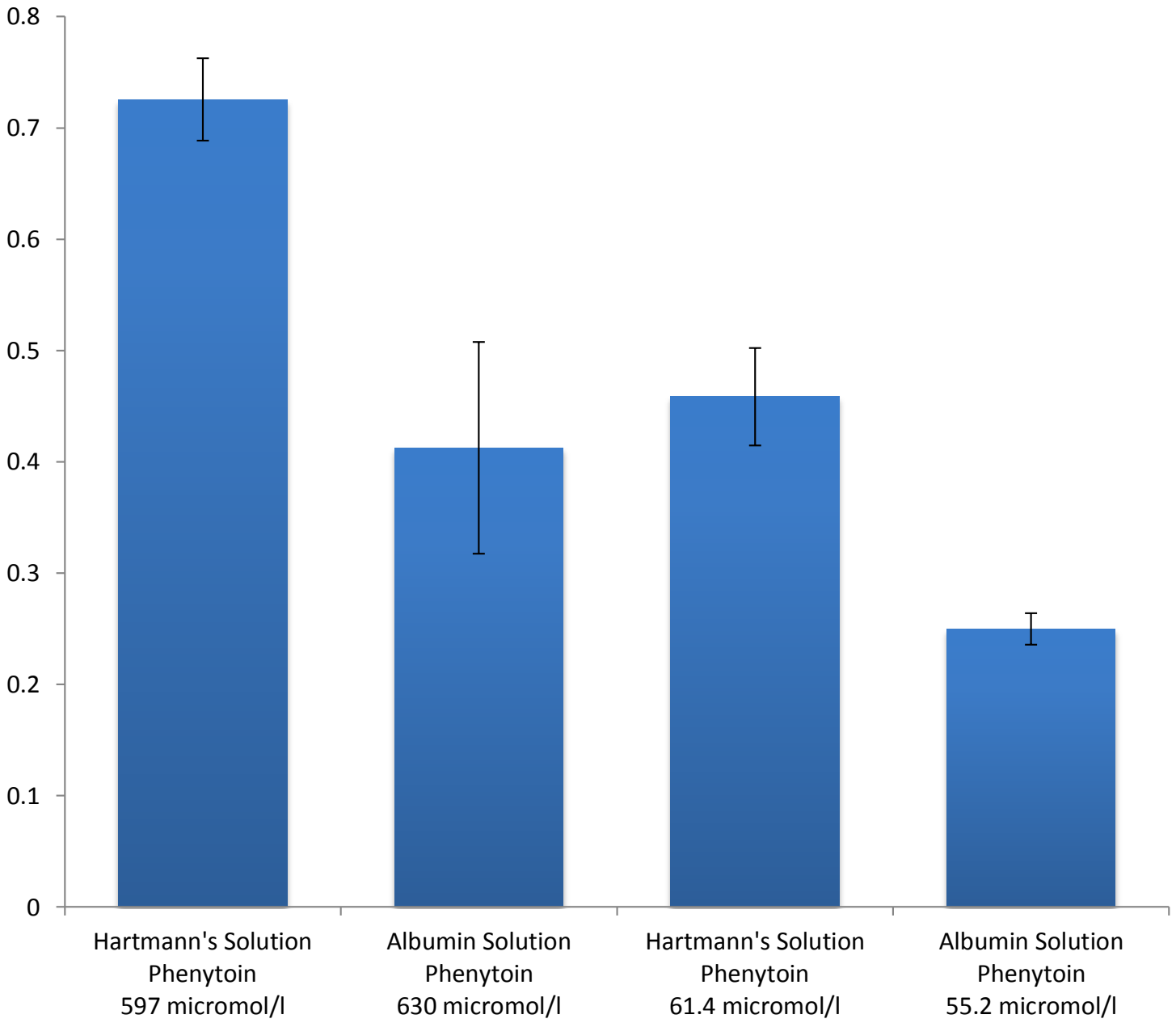
Phenytoin concentrations measured in original samples and thereafter in subnatants following addition of Intralipid in Hartmann's solution with and without Human albumin. Results are expressed as mean values (n = 10)

	Measured initial total phenytoin conc^{n} (μmol.l^{-1})	**Calculated initial free phenytoin conc^{n} (μmol.l^{-1})**	**Measured total conc^{n} of phenytoin remaining in non-lipid compartments (free+albumin) (μmol.l^{-1})**	**Calculated free aqueous phenytoin conc^{n} (μmol.l^{-1})**	**Calculated total conc^{n} of phenytoin removed by lipid (μmol.l^{-1})**	**Calculated fraction of total drug removed by lipid**	**Calculated conc^{n} of phenytoin in lipid (μmol.l^{-1})**	**[Phenytoin] in lipid : [Phenytoin] in non-lipid compartments (free+albumin)**	**[Phenytoin] in lipid : free [Phenytoin]**
Hartmann's Solution	597.2	597.2	163.9	163.9	433.3	0.72	16898.7	1 : 103	1 : 103
Albumin Solution	630.3	63.0	370.2	37.0	260.1	0.41	10142.0	1 : 27	1 : 274
Hartmann's Solution	61.4	61.4	33.3	33.3	28.2	0.45	1097.9	1 : 33	1 : 33
Albumin Solution	55.2	5.5	41.4	4.1	13.8	0.25	537.8	1 : 13	1 : 130

Legends to Figures

Figure 1. Bar graphs showing proportion of sequestration by Intralipid of phenytoin present in high and low concentrations in either Hartmann's or albumin containing solutions compared to the control group (data represents mean +/- SD); note first two bars refer to solutions with high initial phenytoin concentrations (at the values indicated) and last two bars refer to low initial phenytoin concentrations as stated in the method section of the text

Figure 2. Stacked bar graphs showing relative proportion of distributions of phenytoin present in high and low concentrations for two and three compartment phases pre and post addition of Intralipid. Blue bars - phenytoin present in the aqueous compartment (Free phenytoin); Red bars - phenytoin contained in the lipid compartment (Lipid); Green bars - Albumin-bound phenytoin (Albumin)


0.8
0.7
0.6
0.5
0.4
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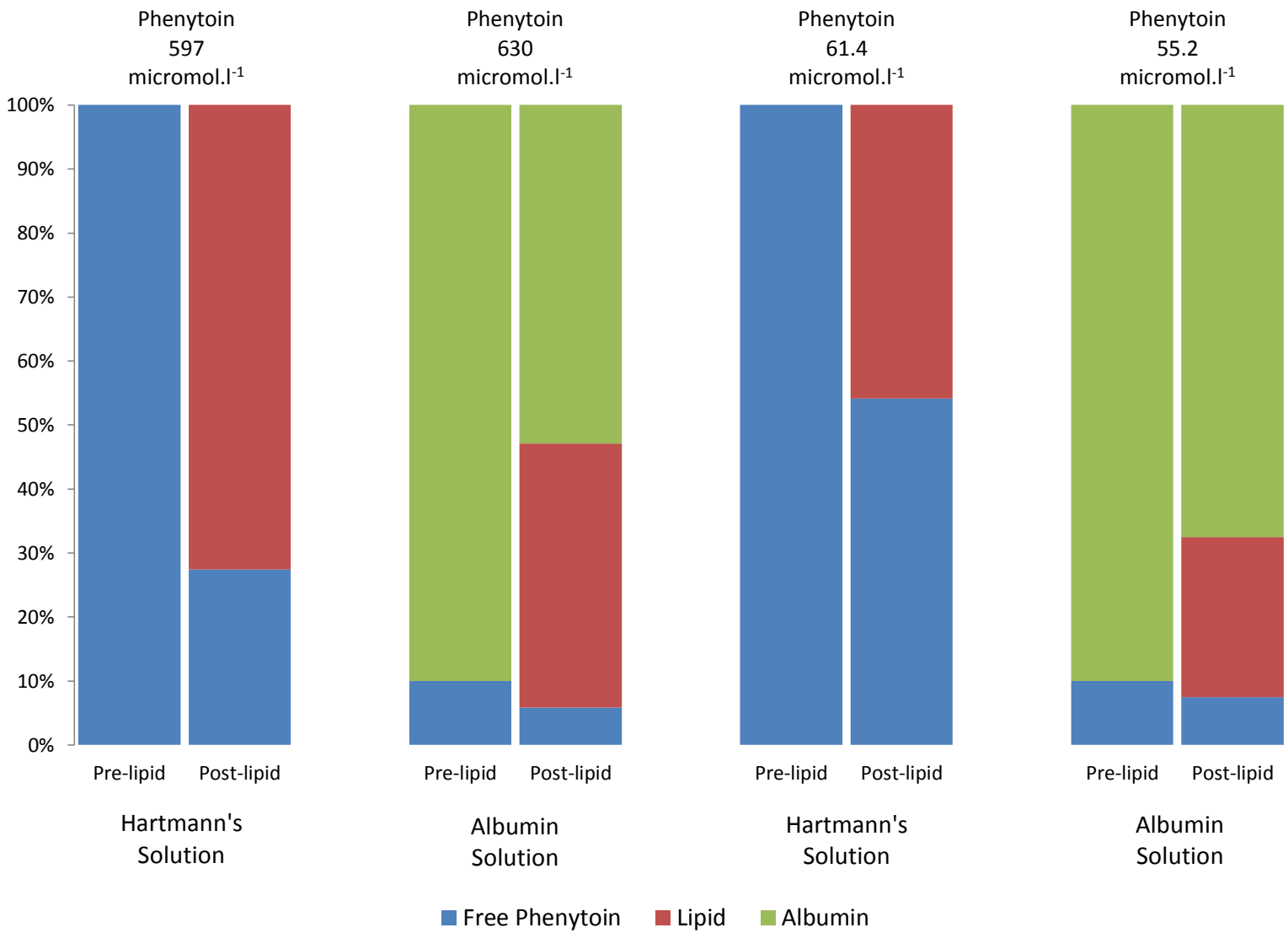
Hartmann's Solution Phenytoin 597 micromol/l
Albumin Solution Phenytoin 630 micromol/l
Hartmann's Solution Phenytoin 61.4 micromol/l
Albumin Solution Phenytoin 55.2 micromol/l


Phenytoin
597
micromol.l-1
Phenytoin
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61.4
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Phenytoin
55.2
micromol.l-1
100%
90%
80%
70%
60%
50%
40%
30%
20%
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Pre-lipid
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