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SAS Audit and Research Prize Submission
January 2013

The influence of carrier protein saturation on the sequestration of free carbamazepine by Intralipid: a model to help explain the utility of lipid emulsion therapy in bupivacaine toxicity.

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The influence of carrier protein saturation on the sequestration of free carbamazepine by Intralipid: a model to help explain the utility of lipid emulsion therapy in bupivacaine toxicity.

Intravenous lipid emulsion (ILE) for the treatment of local anaesthetic systemic toxicity (LAST) has become established in less than ten years. Investigators representing disciplines from anaesthesia, toxicology and elsewhere continue to explore the mechanisms responsible for ILE's effects. Treatment of LAST with ILE involves the re-purposing of a pharmaceutical agent originally developed over fifty years ago to provide intravenous nutritional support. In a sense, this serendipitous finding is both fortuitous and unfortunate in that treatment of LAST with ILE has evolved from anecdote to antidote without the detailed scientific scrutiny usually required for most therapies. Currently uncertainty exists regarding the physiological and pharmacological considerations determining which drugs are amenable to *lipid rescue* and, if so, the most effective dose and rate of delivery of ILE. Lipophilic drug carriage within the intravascular compartment is complex and usually facilitated by plasma proteins including α-1-acid glycoprotein and albumin. However the capacity for drug binding by plasma proteins can be exceeded, especially if the drug in question is present in overdose, which could result in a higher than expected portion of 'free' drug present within the plasma. I examined the lipid sink effect in relation to the degree of carrier protein saturation of the lipophilic drug carbamazepine. The aim was to determine the influence of drug-protein binding on the potential drug sequestration capacity of Intralipid. The hypothesis for this study was that Intralipid would sequester carbamazepine to a greater extent at higher carbamazepine concentrations once carrier protein binding is exceeded. The results derived from this study were employed to facilitate a comparison with the pooled data derived from studies using bupivacaine, to provide insights into the use of ILE for bupivacaine toxicity.

Methods

After consultation, NRES advised the protocol for this laboratory study exempted it from the requirement to obtain formal ethical approval.

Carbamazepine (Hospira, Leamington Spa) was added to differing ratios of Hartmanns solution (Baxter, Thetford) and human albumin (Zenalb, Elstree) to achieve final test tube concentrations (once all necessary additional solutions had been added) of 137, 596, 786 and 1542 $\mu mol.l^{-1}$ of carbamazepine in 450 $\mu mol.l^{-1}$albumin respectively. These concentrations were selected based on the observation that at an albumin concentration of 450 $\mu mol.l^{-1}$ binding site occupancy is complete once the carbamazepine concentration exceeds 600 $\mu mol.l^{-1}$ [1]. Hence these values were chosen to represent carbamazepine concentrations associated with incomplete (i.e. the two lower values) and complete (i.e. the two higher values) carrier protein saturation.

Intralipid (Fresenius, Runcorn) was added to each test tube to emulate the concentration achieved with ILE therapy equivalent to the administration of 500ml Intralipid in a 75 kg subject. Following vigorous mixing and heparin flocculation the samples were decanted into burettes. Following separation overnight, the aqueous subnatant of each sample was drained and the concentrations of carbamazepine measured. Similarly carbamazepine concentrations were measured in identically prepared controls that had not received Intralipid. The free drug concentration in all the specimens was calculated and then the extent of sequestration of free carbamazepine by Intralipid determined.

Results

Carbamazepine sequestration results are summarised in Table 1. At low drug concentrations Intralipid sequestration of carbamazepine is modest, however a sharp increase in the degree of carbamazepine sequestration by Intralipid occurs once the threshold for complete saturation of albumin by carbamazepine is exceeded, coinciding with a clearly measurable increase in the ‘free’ concentration of carbamazepine. Figure 1 represents the same data graphically and

acknowledges the contribution of both protein binding and Intralipid in order to keep 'free' carbamazepine levels low.

Discussion

Carbamazepine was chosen as a surrogate for bupivacaine because it has been shown that its lipid sequestration increases with an increase in the free concentration although the protein bound drug portion is unaffected [2]. Carbamazepine is 75% protein bound to a single site on albumin, making free drug calculation possible at pre and post saturation levels with accuracy [1]. It is unionized at any pH, has lipophilicity between bupivacaine and lignocaine at physiological pH and is easily assayed.

This study confirms the experimental hypothesis, namely that significant drug sequestration by Intralipid occurs once albumin saturation has been exceeded, rendering more 'free' drug available to the lipid sink. Previously it has been reported that the lipid sink effect is relatively poor at low drug concentrations, questioning the value of Intralipid at toxic drug concentrations [3]. This supposition cannot be justified based on my study's findings. Intralipid's utility in the clinical context would be best considered for a lipophilic drug overdose in which the protein saturation concentration is similar to the threshold drug concentration at which signs of toxicity begin to manifest. Fortuitously this appears to be the case with bupivacaine which is primarily bound (with high affinity) to α-1-acid glycoprotein and also to albumin [4]. Table 2 comprises a tabulated summary of other studies reporting free and bound proportions of bupivacaine including the capacity for sequestration by ILE (expressed as a percentage) at those concentrations [3-11]. The accompanying graphical representation (Figure 2) shows comparable characteristics to those identified with carbamazepine in this study.

It is increasingly evident that in the clinical context several physiological variables influence the drug sequestration characteristics of Intralipid. For example bupivacaine exhibits reduced binding to α-1-acid glycoprotein (but not albumin) during acidosis, which is likely to increase free bupivacaine levels in established toxicity [12]. The greater degree of ILE binding observed at higher bupivacaine concentrations might compensate for this element with the additional

safeguard that progressive correction of acidosis will directly increase protein binding and indirectly improve lipophilicity through establishment of a more favourable LogD value. At extremely high drug concentrations the sequestration by Intralipid decreases again [10], possibly when the sequestration capacity of Intralipid is exceeded.

Conclusion

This study supports the potential benefits and use of ILE in the treatment of toxicity caused by bupivacaine. As the concentration of free drug increases after protein saturation occurs, sequestration by the lipid sink appears more effective. The complex mechanism by which the lipid sink sequesters bupivacaine present at toxic concentrations appears to be heavily influenced by protein binding.

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Tables

Table 1. Carbamazepine concentrations measured in original samples and thereafter in subnatants following addition of Intralipid in Hartmanns solution and human albumin with and without Intralipid

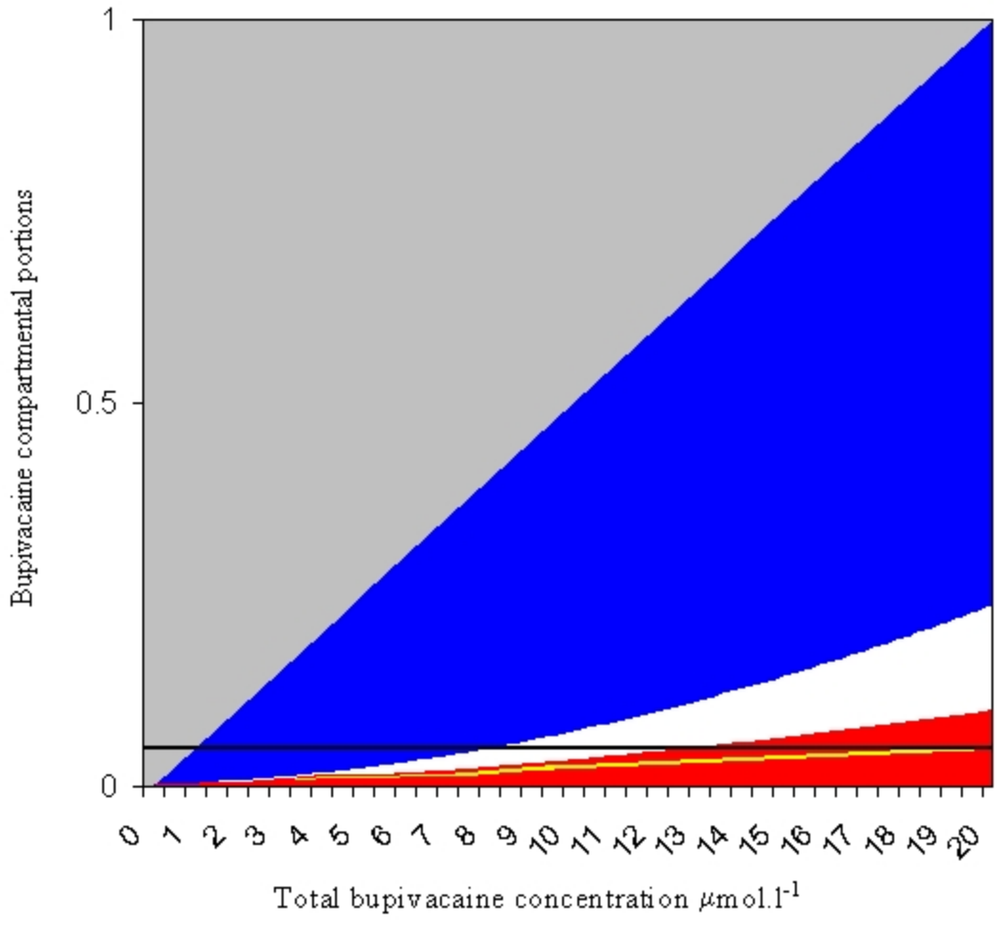
Initial *total* carbamazepine conc ($\mu mol.l^{-1}$)	**Initial *free* carbamazepine conc ($\mu mol.l^{-1}$)**	**Free carbamazepine conc remaining in aqueous phase after sequestration with Intralipid ($\mu mol.l^{-1}$)**	**Free conc of carbamazepine removed by lipid fraction ($\mu mol.l^{-1}$)**	**Portion of carbamazepine removed by lipid**
137	34	29	5	0.14
596	149	115	34	0.21
786	336	186	150	0.45
1542	1092	435	657	0.6

Table 2 Tabulated data derived from published studies related to protein binding and (where indicated) lipid sequestration of bupivacaine .

Source	Total bupivacaine ($\mu mol.l^{-1}$)	Measured protein binding (%)	Non-protein bound bupivacaine concentration derived from quoted source ($\mu mol.l^{-1}$)	Calculated Non-protein bound bupivacaine concentration expected at 95% protein binding ($\mu mol.l^{-1}$)	Lipid sequestration non protein bound bupivacaine (%)
Veering[5]	1.75*	95.2	0.08	0.08	
Litonius [3]	2*	96.5	0.07	0.1	22
Tsen [4]	3.5*	93.5	0.28	0.175	
Dauphin[6]	4*	91.3	0.35	0.2	
Howell [7]	5*	88	0.72	0.25	
Tsen[4]	17.5*	84.8	2.26#	0.875	
Howell [7]	20	72	5.6#	1	
Ruan[8]	35			1.75	37
Howell[7]	35	60	14#	1.75	
Howell[7]	50	50	25#	2.5	
Laine[9]	70	51	34#	3.5	74

Mazoit[10]			250#		75
Ruan[8]	350				50
Mazoit[10]			750#		66
Mazoit[10]			2000#		60
Mazoit[10]			3000#		50

*Total bupivacaine concentrations less than those associated with full saturation of α-1-acid glycoprotein are shaded in grey.
#Free bupivacaine concentrations exceeding the toxic free bupivacaine level, known to be 1 μmol.l^{-1} are shaded in red [See Ref 11]


1
0.5
0
Bupivacaine compartmental portions
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Total bupivacaine concentration µmol.l-1

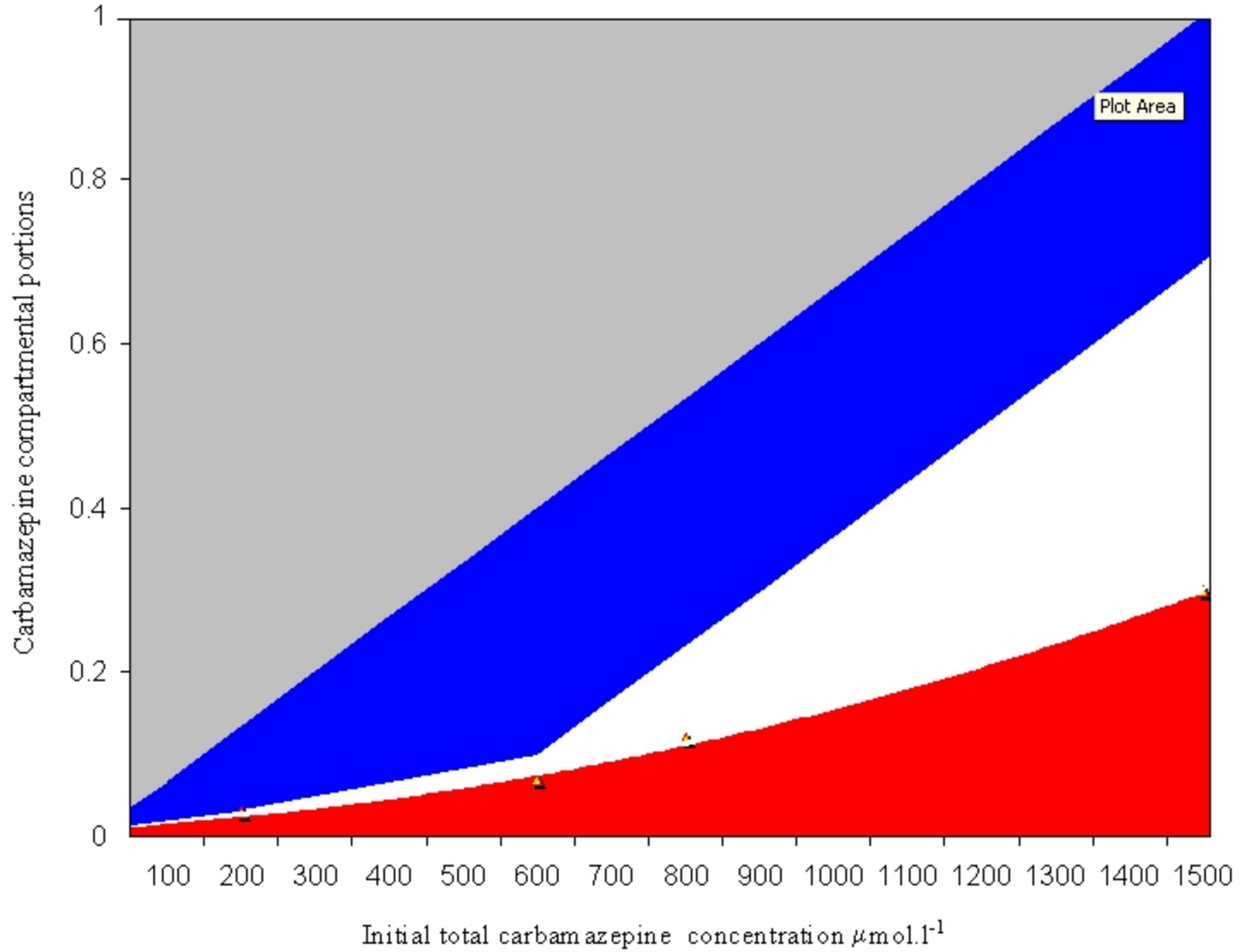

Plot Area
Carbamazepine compartmental portions
1
0.8
0.6
0.4
0.2
0
100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500
Initial total carbamazepine concentration µmol.l-1

Figure captions

Figure 1. Carbamazepine distribution in protein, lipid and aqueous compartments: A graphical presentation derived from the experimental data presented in Table 1.

Legend

Protein bound carbamazepine portion: blue area;

Non-protein bound carbamazepine portion: white and red areas;

Lipid bound carbamazepine portion: white area;

Free remaining aqueous carbamazepine portion: red area;

Figure 2. Theoretical bupivacaine distribution in protein, lipid and aqueous compartments after ILE. A graphical presentation derived from the reference sources described in Table 2.

Legend

Protein bound bupivacaine portion: blue ;

Non-protein bound bupivacaine portion: white and red areas;

Lipid bound Bupivacaine portion: white area;

Free remaining aqueous bupivacaine portion: red area;

Expected non-protein bound bupivacaine portion at 95% protein binding: yellow line;

Black line represents the aqueous free bupivacaine concentration threshold for toxicity (1 $\mu mol.l^{-1}$)