Dr Johann Willers

Associate Specialist in Anaesthesia
Department of Anaesthesia and Intensive Care
Western Sussex Hospitals NHS Trust
Worthing Hospital
Lyndhurst Road
Worthing
West Sussex
BN11 2DH

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Determination of the inhibitory effect of Intralipid on methaemoglobin produced by compounds of different lipid solubilities

J. W. Willers[1]

1. Associate Specialist, Worthing Hospital, Worthing, UK

Correspondence to: Dr Johann Willers, *Department of Anaesthesia and Intensive Care, Worthing Hospital, Lyndhurst Road, Worthing, West Sussex, BN11 2DH.*

Johann.willers@WSHT.nhs.uk

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Intravenous lipid emulsion (ILE) was shown to reduce the toxic effects of chlorpromazine, a lipophilic drug, in a rabbit model almost forty years ago [1]. The originally postulated theory that ILE acted as a 'storage depot' has subsequently evolved into the current concept of an intravascular 'lipid sink'. Evidence for ILE reversal of lipophilic drug toxicity has escalated particularly over the past decade, especially for local anaesthetics [2]. Greater understanding of the role of Intralipid has already prompted the AAGBI to revise its protocol for treatment of local anaesthetic systemic toxicity, however the exact mechanism or mechanisms by which Intralipid exerts it clinical effect has yet to be fully established. It has been suggested that the relative lipid solubilities (log P values) of drugs present in overdose may predict the ability of ILE to bind these drugs and reduce their toxic effects. To date, animal studies and anecdotal reports from humans describing the effects of ILE to reduce the toxic effects of lipophilic drug in overdose have shown most promise with drugs of high log P values such as propranolol, verapamil, amiodarone and clomipramine [2].

Methaemoglobin occurs by oxidation of ferrous ion (Fe^{2+}) found in haemoglobin to its ferric (Fe^{3+}) state. The fraction of haemoglobin within blood present as methaemoglobin can be measured using co-oximetry. Previously Intralipid has been shown to suppress the methaemoglobin formed by Glyceryl Trinitrate, a highly lipid soluble drug, in a dose dependent manner *in vitro* [3]. We hypothesised that the likely suppression by Intralipid of methaemoglobin formation caused by compounds with different log P values would be associated with their respective lipid solubilities. We identified three direct acting methaemoglobin forming compounds of differing lipid solubilities, Glyceryl Trinitrate (GTN), 2-amino-5-hydroxytoluene (AHT) and Sodium Nitrite ($NaNO_2$) (Table 1) and decided to test their effects on whole blood in vitro in the presence or absence of Intralipid.

Methods

Ethical and research governance approval was granted through the Sussex NHS Research Consortium (10/H1101/62, 1365/WSHT/2010). Preliminary work for the study suggested that 15 subjects were required to achieve a power of 80% at a significance level of 0.05 for a specified mean difference of 10% between the control and treatment groups.

Following informed written consent, fresh whole blood was collected from 15 healthy non-smoking volunteers (n=15) who were not taking medication known to cause methaemoglobinaemia. The blood was divided into eight glass test tubes and prepared according to Table 2. Briefly, each compound was added to blood in the presence of 1 ml of 20% Intralipid or 1ml of Hartmann's solution to act as its own control. Two further controls contained Hartmann's solution and/or Intralipid but no methaemoglobin forming compound. The individual concentration of each methaemoglobin forming compound (Glyceryl Trinitrate 5mg.ml^{-1}, Sodium Nitrite 1mg.ml^{-1}, 2-amino-5-hydroxytoluene 2.5mg.ml^{-1}) was chosen to produce a final methaemoglobin concentration of approximately 5g.dl^{-1}. All test tubes were maintained at 37 ºC and regularly mixed. Samples of 0.2 ml were taken 30 and 60 minutes from the start of the experiment and analysed using a Radiometer ABL 725 to determine the percentage of methaemoglobin (% MetHb) present. Methaemoglobin concentrations were calculated from the % MetHb and the Hb concentration (g.dl^{-1}) derived from the blood gas analyser.

All data was analysed using the open source statistical package R (GNU project, R Foundation for Statistical Computing, Vienna, Austria). A Wilcoxon signed rank test was used to compare the control groups with the treatment groups individually and also for comparing within each paired two sample group. A p value < 0.05 was considered statistically significant.

Handling and disposal of all blood samples was in accordance with local hospital policies and guidelines.

Results

In keeping with the design of this study, the doses of GTN, AHT and $NaNO_2$ selected produced methaemoglobin with an approximate concentration of $5g.dl^{-1}$. Addition of Intralipid to the $NaNO_2$ group had no effect on the formation of methaemoglobin compared its corresponding Hartmann's control ($p > 0.5$; Figure 1). Addition of Intralipid to the AHT treated group produced a small but insignificant reduction ($p > 0.5$; Figure 2) in methaemoglobin formed, whereas addition of Intralipid to the GTN group resulted in a 75% and 60% reduction in methaemoglobin formation at 30 and 60 minutes respectively ($p < 0.001$; Figure 3). Both Hartmann's and Intralipid control groups did not produce any methaemoglobin at 30 or 60 minutes.

Discussion

It has been established that Intralipid suppresses the methaemoglobin forming ability of GTN in a dose dependent manner [3]. Results from this study demonstrate that whereas addition of Intralipid has the capacity to suppress methaemoglobin formation resulting from the lipophilic drug GTN, this capacity is not demonstrated in the presence of drugs that possess low lipid solubility (i.e. AHT) or are virtually lipid insoluble (i.e. $NaNO_2$). In these conditions, methaemoglobin formation is unaltered by the presence or absence of Intralipid. The dose of Intralipid used in this study was chosen to approximate to the final concentration of Intralipid likely to occur in clinical settings in which 'Lipid Rescue' therapy has been deployed.

Conclusion

This work provides strong evidence to advance the concept of the lipid sink theory in that the greatest binding effect of Intralipid occurs with compounds possessing high log P values. This may explain reports describing greater apparent benefit from Intralipid administered in overdoses involving lipophilic drugs and help establish suitable criteria governing the appropriate deployment of intravenous lipid emulsions in the treatment of drug overdoses.

References

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Table 1 Choice of methaemoglobin forming agents based upon Log P values

Drug/compound	Lipid solubility	Log P
Glyceryl Trinitrate (GTN)	Highly lipid soluble	2.22
2-amino-5-hydroxytoluene (AHT)	Partially lipid soluble	0.17
Sodium Nitrite ($NaNO_2$)	Virtually lipid insoluble	-3.7

Table 2 Description of individual test tube preparation in this study. Abbreviations: **GTN** (Glyceryl Trinitrate 5 $mg.ml^{-1}$), **$NaNO_2$** (Sodium Nitrite 1 $mg.ml^{-1}$), **AHT** (2-amino-5-hydroxytoluene 2.5 $mg.ml^{-1}$)

Group	Blood volume (ml)	Hartmann's solution volume (ml)	Intralipid* volume (ml)	GTN volume (ml)	$NaNO_2$ volume (ml)	AHT volume (ml)	Total volume (ml)	Final Intralipid concentration ($mg.ml^{-1}$)
Hartmann's control	7	2	0	0	0	0	9	**0**
Intralipid control	7	1	1	0	0	0	9	**22**
GTN + Hartmann's solution	7	1	0	1	0	0	9	**0**
GTN + Intralipid	7	0	1	1	0	0	9	**22**
$NaNO_2$ + Hartmann's solution	7	1	0	0	1	0	9	**0**
$NaNO_2$ + Intralipid	7	0	1	0	1	0	9	**22**
AHT + Hartmann's solution	7	1	0	0	0	1	9	**0**
AHT + Intralipid	7	0	1	0	0	1	9	**22**

*20% Intralipid employed in initial test tube preparation

Figure 1. – Methaemoglobin concentrations ($g.dl^{-1}$) at 30 and 60 minutes following addition of Sodium Nitrite ($NaNO_2$) to Hartmann's control (white bars) and Intralipid treatment group (grey bars) demonstrating no difference between groups ($p > 0.5$). Mean values ± SEM.

Figure 2. – Methaemoglobin concentrations ($g.dl^{-1}$) at 30 and 60 minutes following addition of 2-amino-5-hydroxytoluene (AHT) to Hartmann's control (white bars) and Intralipid treatment group (grey bars) demonstrating no difference between groups ($p > 0.5$). Mean values ± SEM.

Figure 3. – Methaemoglobin concentrations ($g.dl^{-1}$) at 30 and 60 minutes following addition of Glyceryl Trinitrate (GTN) to Hartmann's control (white bars) and Intralipid treatment group (grey bars). A significant reduction in methaemoglobin concentration is observed in the Intralipid treatment group compared to the Hartmann's control group ($p < 0.001$). Mean values ± SEM.