Intravenous lipid emulsion for treatment of local anaesthetic and tricyclic antidepressant toxicity

Juho A. Heinonen

ACADEMIC DISSERTATION

To be publicly discussed, with the permission of the Faculty of Medicine of the University of Helsinki, in the Auditorium XIV of the University of Helsinki, Unioninkatu 34, Helsinki, on March 19th, 2016, at 10 a.m.

Helsinki 2016
SUPERVISORS:
Erik Litonius, MD, PhD
Department of Anesthesia and Perioperative Care
University of California
San Francisco, CA, USA

Professor Janne T. Backman, MD, PhD
Department of Clinical Pharmacology
University of Helsinki
Helsinki, Finland

REVIEWERS:
Professor Riku Aantaa, MD, PhD
Department of Anaesthesiology, Intensive Care, Emergency Care and Pain Medicine
University of Turku
Turku, Finland

Professor emeritus Pauli Ylitalo, MD, PhD
Clinical Pharmacology and Toxicology
School of Medicine
University of Tampere
Tampere, Finland

OPPONENT:
Docent Kai Knudsen, MD, PhD
Department of Anaesthesia and Intensive Care
Sahlgrenska University Hospital
Gothenburg, Sweden

Dissertationes Scholae Doctoralis Ad Sanitatem Investigandam Universitatis Helsinkiensis

ISSN 2342-3161 (print)

ISSN 2342-317X (online)

Hansaprint
Helsinki, 2016
Finland
To my wife Veera
TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS 6
ABBREVIATIONS 7
ABSTRACT 9
TIIVISTELMÄ 11
INTRODUCTION 13
REVIEW OF THE LITERATURE 15
  Local anaesthetics 15
  Tricyclic antidepressants 19
  Intravenous lipid emulsion 22
  Lipid rescue 23
PURPOSE OF THE STUDY 36
METHODS 37
  Questionnaire study (I) 37
  Human volunteer study (II) 37
  Animal studies (III–V) 38
  Blood and tissue sample handling 42
  Statistical analyses 43
RESULTS 45
  Questionnaire study 45
  Plasma drug concentrations 46
  Tissue drug concentrations 48
  Haemodynamics and ECG 49
  Arterial blood gases 50
  Mitochondrial respirometry 52
  Central nervous system toxicity 52
LIST OF ORIGINAL PUBLICATIONS
This thesis is based on the following publications:


The publications are referred to in the text by their Roman numerals. The original publications are reproduced with the permission of the copyright holders.
ABBREVIATIONS

AAGBI  Association of Anaesthetists of Great Britain and Ireland
ADP   Adenosine diphosphate
ARDS  Acute respiratory distress syndrome
ASRA  American Society of Regional Anesthesia and Pain Medicine
ATP   Adenosine triphosphate
AUC   Area under the concentration-time curve
Bupi  Bupivacaine
BE    Base excess
BP    Blood pressure
cAMP  Cyclic adenosine monophosphate
CARPA Complement activation related pseudoallergy
Coca  Cocaine
Cl    Systemic clearance
CNS   Central nervous system
CV    Cardiovascular
CVP   Central venous pressure
CYP   Cytochrome P450
DDD   Daily defined dose
ECG   Electrocardiogram
EEG   Electroencephalography
EF    Ejection fraction
eNOS  Endothelial nitric oxide synthase
EtCO2 End-tidal carbon dioxide
ETS   Electron transfer system
FCCP  Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone
FiO2  Fraction of inspired oxygen
G     Glutamate
GCS   Glasgow Coma Scale score
i.o.  Intraosseous
IQR   Interquartile range
i.v.  Intravenous
IVRA  Intravenous regional anaesthesia
Lido  Lidocaine
LAST  Local anaesthetic systemic toxicity/intoxication
LBupi Levobupivacaine
LCT   Long-chain triglycerides
logD  Logarithm of octanol:water partition coefficient at physiological pH
logP  Logarithm of octanol:water partition coefficient at pH 7
M     Malate
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>MBRT</td>
<td>Mean body residence time</td>
</tr>
<tr>
<td>MCT</td>
<td>Medium-chain triglycerides</td>
</tr>
<tr>
<td>Mepi</td>
<td>Mepivacaine</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>$P_a$CO$_2$</td>
<td>Arterial carbon dioxide partial pressure</td>
</tr>
<tr>
<td>$P_a$O$_2$</td>
<td>Arterial oxygen pressure</td>
</tr>
<tr>
<td>Prilo</td>
<td>Prilocaine</td>
</tr>
<tr>
<td>PEA</td>
<td>Pulseless electrical activity</td>
</tr>
<tr>
<td>pK$_a$</td>
<td>Dissociation constant</td>
</tr>
<tr>
<td>POPC</td>
<td>1-palmitoyl-2-oleyl-$sn$-glycero-3-phosphocholine</td>
</tr>
<tr>
<td>POPG</td>
<td>1-palmitoyl-2-oleyl-$sn$-glycero-3-(phospho-rac-[1-glycerol])</td>
</tr>
<tr>
<td>Ropi</td>
<td>Ropivacaine</td>
</tr>
<tr>
<td>ROSC</td>
<td>Return of spontaneous circulation</td>
</tr>
<tr>
<td>Rot</td>
<td>Rotenone</td>
</tr>
<tr>
<td>RPP</td>
<td>Rate-pressure product</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SpO$_2$</td>
<td>Peripheral oxygen saturation</td>
</tr>
<tr>
<td>SR</td>
<td>Sinus rhythm</td>
</tr>
<tr>
<td>Suc</td>
<td>Succinate</td>
</tr>
<tr>
<td>SVRI</td>
<td>Systemic vascular resistance index</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>Half-time (describing the decrease of drug concentration by 50% though both elimination and tissue distribution)</td>
</tr>
<tr>
<td>TAP</td>
<td>Transversus abdominis plane</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricyclic antidepressant</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
</tr>
<tr>
<td>V$_d$</td>
<td>Volume of distribution</td>
</tr>
<tr>
<td>VF</td>
<td>Ventricular fibrillation</td>
</tr>
<tr>
<td>VT</td>
<td>Ventricular tachycardia</td>
</tr>
</tbody>
</table>
Abstract

Drug overdoses and poisonings are global health problems resulting in several thousands of deaths annually. In out-of-hospital setting, one of the most common causes of death is an overdose of tricyclic antidepressant, such as amitriptyline. In the hospital, on the other hand, local anaesthetic systemic toxicity is one of the most feared and potentially a life-threatening complication. Both tricyclic antidepressants and local anaesthetics lack specific antidotes. However, as they all are lipophilic drugs, intravenously administered lipid emulsion has been suggested as a potential treatment for both intoxications. Originally, the proposed mechanism of action of lipid emulsion was a lipid sink that entraps lipophilic drugs and prevents their action in target tissues. Nowadays, lipid emulsion is a recommended treatment for local anaesthetic systemic toxicity in, for instance, the UK and the US in spite of the fact that its actual mechanisms of action and benefit remain uncertain.

In the first study of this thesis, the incidence of local anaesthetic systemic toxicity and the adoption rates of lipid emulsion treatment in Finnish anaesthesia departments were elucidated (I). The other studies of this thesis investigate the efficacy of intravenously administered lipid emulsion in both local anaesthetic toxicity and amitriptyline. The effect on lidocaine induced central nervous system toxicity were studied in human volunteers (II). The effect of lipid emulsion on levobupivacaine intoxication in a situation simulating seizures (III), on the tissue distribution of amitriptyline (IV), and on mitochondrial respiration in bupivacaine cardiac toxicity (V) were studied in pigs. In each of these studies an assessment of the degree of the entrapment of the drug by intravenous lipid emulsion was included.

The incidence of local anaesthetic systemic toxicity in Finland is low, only 0.7 per 10,000 regional anaesthesias (I). Lipid emulsion treatment is adopted to less than half of the Finnish anaesthesia departments. In human volunteers, lipid emulsion does not affect the electroencephalography changes or the subjective symptoms caused by lidocaine. Lidocaine was also not entrapped into plasma, but its volume of distribution was slightly increased (II). In pigs, lipid emulsion has no effect on levobupivacaine intoxication which is exacerbated by acidosis and hypoxaemia as measured by reversing of electrocardiogram and haemodynamics from toxic changes (III). Levobupivacaine was also not entrapped into plasma. When lipid emulsion was infused in amitriptyline intoxication, amitriptyline was slightly entrapped into circulation and the brain amitriptyline concentration was reduced by 25% (IV). After higher lipid emulsion dose than recommended, recovery from bupivacaine cardiac toxicity was improved through peripheral vasoconstriction (V). Cardiac mitochondrial respiration was also slightly improved at the same time, and bupivacaine was slightly entrapped into plasma.

To conclude, this is the first Finnish study to show that the incidence of local anaesthetic toxicity is very low: 0.7 per 10,000. Lipid emulsion can reduce amitriptyline brain concentration but has no effect on local anaesthetic systemic toxicity if used in clinically
recommended doses. If a higher dose is administered, lipid emulsion improves recovery from local anaesthetic toxicity through peripheral vasoconstriction in pigs. The safety of the higher dose to men remains, however, unknown.


Yhteen vetona voidaan todeta, että tämä oli ensimmäinen suomalainen tutkimus, jossa osottettiin puudutemyrkyksen olevan harvinainen komplikaatio: alle 1 tapaus 10.000 puudutusta kohden. Antamalla rasvaemulsiota voidaan alentaa aivojen
amitriptyliinipitoisuutta, mutta puudutemyrkyksen oireisiin sillä ei ole vaikutusta hoitosuositukseen mukaisena annoksena. Toisaalta käyttämällä suurempaa annosta voidaan ainakin porsasmallissa tehostaa toipumista puudutemyrkykyksestä aiheuttamalla verenkierron supistuminen. Suuremman rasvaemulsioannoksen turvallisuudesta ihmisillä ei kuitenkaan ole tietoa.


**INTRODUCTION**

Today, drug overdoses are a global problem thought to have caused up to 183,000 deaths among working aged persons worldwide in 2012 (United Nations Office on Drugs and Crime, 2014). In Finland, every year drug overdoses cause more than 1,000 deaths (Vuori et al., 2012). Self-poisonings are not the only cause of drug related emergencies or deaths; iatrogenic drug administration for therapeutic purposes can cause life-threatening situations and even deaths. For example, local anaesthetics are mainly used to produce local anaesthesia but their systemic effects can be life threatening. This local anaesthetic systemic toxicity or intoxication (LAST) is a consequence of an unintentional injection of local anaesthetic directly into the bloodstream (de Jong, 1978), or of the absorption of the local anaesthetic after administration of an overdose which may have been used in epidural or brachial plexus blocks for surgical anaesthesia (Di Gregorio et al., 2010). Untreated, the complication may be fatal (Lee et al., 2008).

Next after opioids, the drug most commonly associated with deaths by overdose is the tricyclic antidepressant (TCA) amitriptyline (Vuori et al., 2012). Typically these poisonings happen to persons of working age and are intentional (Peiris-John et al., 2014; Vuori et al., 2012). Unfortunately, the cause of death in these suicides is typically prescription medication (Sinyor et al., 2012). Even though almost 90% of poisoning deaths occur outside of hospital (Bjornaaas et al., 2010), hospitalisations due to drug overdoses are also an increasing problem, with over 100 hospitalisations per 100,000 persons per year in some parts of the United States (US) (Slavova et al., 2014). Fortunately, if the patient is reached in time the prognosis is usually good, as up to 94% of the hospitalised poisoning patients can be discharged within the next 24 hours, and only a minority needs treatment in an intensive care unit (Lapatto-Reiniluoto et al., 1998).

Local anaesthetics and TCAs are both lipophilic drugs (ChemSpider, 2015) without specific antidotes. The treatment of both LAST and TCA poisoning has been symptomatic. In case of LAST, the treatment includes securing the airways, normalisation of blood pH, treating seizures, managing haemodynamic instability and treating electrocardiogram (ECG) abnormalities possibly with sodium bicarbonate (McLeod et al., 2008). Treatment of TCA poisonings is quite similar, except that for preventing gastrointestinal absorption of TCA, activated charcoal is administered intragastrically (Body et al., 2011).

The hypothesis that life-threatening poisonings caused by lipophilic substances could be treated with a large dose of intravenous (i.v.) lipid emulsion originated when Weinberg and colleagues (1998) noted that pre-treatment with intravenous lipid emulsion increased the bupivacaine dose required to produce asystole in rats. Eight years later, the first case reports of treating LAST with lipid emulsion appeared (Litz et al., 2006; Rosenblatt et al., 2006) and eventually the Association of Anaesthetists of Great Britain and Ireland (AAGBI) and the American Society of
Regional Anesthesia and Pain Medicine (ASRA) decided to recommend intravenous lipid emulsion for the treatment of LAST (Cave et al., 2010; Neal et al., 2010). Later, animal studies (Harvey and Cave, 2007) and case reports (Engels and Davidow, 2010) of lipid rescue of also TCA poisoning were published. As a consequence, lipid emulsion is now added as a possible adjuvant treatment of TCA poisoning (Body et al., 2011). After more than 15 years of the first lipid rescue studies and more than 60 case reports, the lipid rescue is not evidence based and the possible mechanisms of action of lipid emulsion still remain unresolved (Harvey and Cave, 2014; Tucker, 2014).

Therefore, the aim of this thesis was to figure out the incidence of LAST in Finland and to investigate the effects of lipid emulsion in the treatment of LAST and amitriptyline poisonings. It was hypothesised that lipid emulsion could alleviate central nervous system (CNS) toxicity of local anaesthetics, reduce amitriptyline concentration in tissues, and improve recovery of bupivacaine cardiac toxicity by increasing the mitochondrial respiration.
Review of the literature

Local Anaesthetics
The development of local anaesthesia dates back to the middle of the 19th century when German chemist Niemann (1860) managed to isolate the active ingredient of coca leaf naming it cocaine. The modern age of local anaesthesia began, however, in the 1940s when Swedish chemist Löfgren with his group synthesised the first modern local anaesthetic, lidocaine (Löfgren and Lundqvist, 1947), which was introduced into clinical use by the end of the decade (Gordh, 1949). The development of modern local anaesthetics continued in Sweden, and long acting local anaesthetics mepivacaine and bupivacaine, which are still in use, were synthesised during the next decade (af Ekenstam et al., 1957) and soon used also clinically. Even though synthesised at the same time as other long acting local anaesthetics, the less toxic ropivacaine and levobupivacaine were introduced into clinical practise much later, in 1996 and by the beginning of 2000s, respectively, being the newest local anaesthetics (Whiteside and Wildsmith, 2001). Today, local anaesthetics are widely used at every level of our healthcare system. In primary health care, the most used local anaesthetic is lidocaine (Figure 1) to provide anaesthesia, for example, for small surgical procedures, such as removal of a naevus. Long acting local anaesthetics, such as bupivacaine (Figure 2), can provide sufficient anaesthesia even for surgery and childbirth. Unfortunately, not all physicians using local anaesthetics respect, or possibly even are aware of, the potential risks of local anaesthetics (Rao et al., 1999; Sagir and Goyal, 2014; Scherrer et al., 2013).

![Figure 1. Chemical structure of lidocaine.](image1)

Pharmacology
Local anaesthetics are amine molecules that can be divided into esters (procaine type; Figure 3) and amides (lidocaine type; see Figure 1) according to the binding type between the lipophilic and hydrophilic portions. Both types of local anaesthetics are lipophilic and weak bases with a pKₐ (pH at which molecules are half-dissociated) near 8 (Table 1). Therefore, in

![Figure 2. Chemical structure of bupivacaine.](image2)

![Figure 3. Chemical structure of procaine.](image3)
an acidic environment the molecules become ionised which increases their hydrophilicity (Figure 4). To increase their hydrophilicity and solubility for clinical use, local anaesthetics are typically formulated as hydrochloride salts.

The main mechanism of action of local anaesthetics is the blockade of voltage-gated sodium channels (Figure 5) which are situated within the cell membrane of, for instance, axons and cardiomyocytes (Hille, 1977). The presence of a local anaesthetic blocks the ion channel pore and prevents membrane depolarisation by preventing the fast flow of Na\(^+\) ions into the cell (Catterall, 2012). The binding site in these sodium channels lies on the inner side of the cell membrane (Catterall, 2012). To reach their binding site the local anaesthetics have to cross the cell membrane in the lipophilic uncharged form. After entering the cell, local anaesthetics become ionised due to the acidic intracellular environment and are able to bind to the sodium channel (Hille, 1977). At that time, the channels are preferably in either open or inactive state, and therefore, accessible through the open activation gate (Catterall, 2012; Hille, 1977); in the resting state of sodium channel the affinity of the local anaesthetic to the channel is weaker (because the activation gate is closed) with the exception of bupivacaine which slowly

| Table 1. Physicochemical and some pharmacokinetic properties of local anaesthetics |
|---------------------------------|-------------|-------------|---------------|-----------------|----------------------------|
|                                 | pK\(_a\) at | LogD        | LogP         | V\(_d\) (l kg\(^{-1}\)) | Plasma protein binding (%) | Approximate anaesthesia duration (min) |
| Esters                          | 25\(^\circ\)C|             |              |                             |                           |                                      |
| Chlorprocarine                  | 9.3         | 1.15        | 2.93         | 0.50                        | 92                        | 45                                    |
| Cocaine                         | 8.6         | 1.22        | 3.08         | 1.96                        | 70                        | 100                                   |
| Amides                          |             |             |              |                             |                           |                                       |
| Lidocaine                       | 7.8         | 1.80        | 1.66         | 1.30                        | 70                        | 100                                   |
| Prilocaine                      | 8.0         | 1.33        | 1.74         | 2.73                        | 55                        | 100                                   |
| Mepivacaine                     | 7.9         | 1.40        | 2.04         | 1.2                         | 80                        | 100                                   |
| Ropivacaine                     | 8.2         | 2.32        | 3.11         | 0.84                        | 94                        | 150                                   |
| Bupivacaine                     | 8.2         | 2.68        | 3.64         | 1.02                        | 95                        | 175                                   |
| Levobupivacaine                 | 8.2         | 2.68        | 3.64         | 0.9                         | 95                        | 175                                   |

pK\(_a\), dissociation constant (Strichartz et al., 1990; Wiczling et al., 2006); logD, logarithm of predicted octanol:water partition coefficient at physiological pH (ChemSpider, 2015); logP, logarithm of predicted octanol:water partition coefficient at pH 7 (ChemSpider, 2015); V\(_d\), volume of distribution (Chow et al., 1985; Rosenberg et al., 2004); protein binding (Bachmann et al., 1990; Burm et al., 1994; Edwards and Bowles, 1988; Lee et al., 1989; Tucker and Mather, 1975); anaesthesia duration of rat sciatic nerve blocking (Mather and Tucker, 2008).

Figure 4. Ionisation of lipophilic lidocaine base into its hydrophilic acid form.
Figure 5. Structure and normal cycle of voltage-gated sodium channels during action potential: closed channel opens during action potential allowing rapid Na$^+$ influx, but rapidly inactivates ending Na$^+$ flow (above). On the left and below, the local anaesthetic base (L) permeates the cell membrane and ionises in the cytoplasm, after which it is able to bind to a receptor site in the pore of the open or inactivated channel. In the resting state, the activation gate is closed, which prevents the local anaesthetic from binding to the receptor site. The unionised form (especially bupivacaine) can also affect the channel in its resting state through the lipid phase of the cell membrane (Catterall, 2012). Modified from Mantegazza et al. (2010).

Dissociates from the resting state channels (Clarkson and Hondeghem, 1985). In addition to voltage-gated sodium channels, local anaesthetics also interact with several other types of ion channels and G-protein receptors (Hollmann et al., 2001; Komai and McDowell, 2001).

The absorption rate of local anaesthetic into the bloodstream depends on the drug itself, the dosage, addition of vasoconstrictor, and the site of injection, its vascularity and content of fat capable to bind the drug (Tucker and Mather, 1979). Typically, the absorption rate, from highest to lowest, is similar between different agents: intercostal, caudal, epidural, brachial plexus, and sciatic/femoral block (Tucker, 1986). After systemic absorption local anaesthetics are mostly bound to serum proteins, primarily to $\alpha_1$-acid glycoprotein but also to albumin (Mazoit and Dalens, 2004). The fraction of unbound drug increases with increasing total drug concentration (Tucker et al., 1970), as the drug binding proteins become saturated. The pharmacological activity, including the systemic toxicity, of a drug is mainly related to its unbound concentration (Tucker and Mather, 1975), which varies from approximately 5 to 45% between the local anaesthetics (see Table 1, page 16).

Only a small proportion of amide local anaesthetic agents are excreted unchanged (Boyes, 1975; Keenaghan and Boyes, 1972). The metabolism takes place mainly in the liver by the cytochrome P450-dependend mixed function oxidase system (Imaoka et al., 1990; Oda et al., 1995).
After metabolism the different metabolites are then secreted into urine in varying quantities (Boyse, 1975). Urine pH acidification increases the amount of excretion of unchanged agents (Eriksson, 1966; Friedman et al., 1982). Amide local anaesthetics have relatively short terminal elimination half-lives varying from 1.6 h for lidocaine to 2.7 h for bupivacaine (Table 2).

In contrast to amide type local anaesthetics, the ester type local anaesthetics are rapidly hydrolysed by plasma pseudocholinesterases, red cells and liver esterases (Tucker, 1986). Therefore, their half-lives are extremely short: the half-life of chloroprocaine, for instance, in whole blood is only 21–25 s in vitro (O'Brien et al., 1979). As the hydrolysis begins even before the distribution phase, they are relatively safe to use in large doses without a substantial risk of LAST (Rosenberg et al., 2004). Similarly to amide type agents, also the metabolites of ester type local anaesthetics are excreted into urine (O'Brien et al., 1979).

Local anaesthetic systemic toxicity
Local anaesthetic systemic toxicity, or LAST, is the systemic effect of a too high local anaesthetic blood concentration (de Jong, 1978). The typical reason for this high circulating concentration is an unintentional intravascular injection. Other mechanisms are rapid absorption from a highly vascularised injection site or the use of an excessive dose of local anaesthetic. Such potentially toxic cumulative doses occur, for example, when continuous local anaesthetic infusions are used in patients with slow drug metabolism and/or saturated metabolic pathways, or due to technical dosing errors (Whiteman and Kushins, 2014). Chronic diseases, such as renal failure, may alter the pharmacokinetics of local anaesthetics by increasing their uptake and decreasing their clearance (Pere et al., 2003). The toxic effects result from too high local anaesthetic concentrations, and therefore, too much ion channel blockade in the central nervous system (CNS) and heart. As mentioned previously, the free, pharmacologically active, fraction increases as the local anaesthetic concentration rises in the circulation. Therefore, the toxic effects do not appear linearly, since the active fraction is larger at higher concentrations (Tucker and Mather, 1975).

Table 2. Elimination of the clinically most important amide local anaesthetics

<table>
<thead>
<tr>
<th></th>
<th>Cl (l min⁻¹)</th>
<th>E_H</th>
<th>t₁/₂ (h)</th>
<th>MBRT (h)</th>
<th>CYP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>0.95</td>
<td>0.65</td>
<td>1.6</td>
<td>1.6</td>
<td>1A2, 3A4</td>
</tr>
<tr>
<td>Mepivacaine</td>
<td>0.78</td>
<td>0.52</td>
<td>1.9</td>
<td>1.8</td>
<td>?</td>
</tr>
<tr>
<td>Ropivacaine</td>
<td>0.73</td>
<td>0.49</td>
<td>1.9</td>
<td>1.4</td>
<td>1A2, 3A4</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>0.58</td>
<td>0.38</td>
<td>2.7</td>
<td>2.1</td>
<td>3A4, 2C19, 2D6</td>
</tr>
<tr>
<td>Levobupivacaine</td>
<td>0.47</td>
<td>0.67</td>
<td>1.8</td>
<td>2.2</td>
<td>1A2, 3A4</td>
</tr>
</tbody>
</table>

Cl, systemic clearance; E_H, estimated hepatic extraction ratio; t₁/₂, terminal elimination half-life; CYP, metabolising cytochrome P450 enzymes; MBRT, mean body residence time. (Data derived from: Bargetzi et al., 1989; Ekström and Gunnarsson, 1996; Foster and Markham, 2000; Gantenbein et al., 2000; Mather and Tucker, 2008).
The first signs of LAST include numbness, lightheadedness and tinnitus (Foldes et al., 1960; Scott, 1975; 1986). If the systemic local anaesthetic concentration rises, visual disturbances, slurring of speech and muscle twitches follow (Foldes et al., 1960; Scott, 1975; 1986). With even higher concentrations, local anaesthetics cause tonic-clonic seizures and coma (Scott, 1986; Usubiaga et al., 1966). Eventually, apnoea and cardiac depression with arrhythmias follow (Kotelko et al., 1984; Scott, 1986). The reported incidence of LAST in the past 15 years has been 0.6 to 3.5 per 10,000 procedures after excluding spinal anaesthesias (Auroy et al., 2002; Barrington and Kluger, 2013; Ecoffey et al., 2014; Sites et al., 2012), and the majority of cases has been central nervous system intoxications.

The cardiac effects of bupivacaine differ from other local anaesthetics. Lidocaine both enters and exits the cardiomyocytes and sodium channel pores rapidly, and therefore, the myocytes recover quickly from the ion channel blockade during diastole. This rapid dissociation during diastole means that the cardiotoxic effects of local anaesthetics are mitigated by a lower heart rate. Bupivacaine, however, dissociates slowly from the resting sodium channels and also exits the cell more slowly (Clarkson and Hondeghem, 1985). Thus, the cardiac effects of bupivacaine accumulate even at lower heart rates, while the effects of lidocaine can be seen only at high heart rate (Clarkson and Hondeghem, 1985). Bupivacaine has also more potent depressive effect on atrioventricular node conduction (Komai and Rusy, 1981), velocity of action potentials and myocardial contractility (Shibuya et al., 1993) than, for instance, lidocaine (Courtney, 1984). Levobupivacaine, the less cardiotoxic ‘S(−)’ enantiomer of bupivacaine, has less affinity for cardiac sodium channels and the dissociation is faster than with dextrobupivacaine (McClellan and Spencer, 1998), and has, therefore, partially replaced the racemic bupivacaine in clinical use (Whiteside and Wildsmith, 2001).

Part of the local anaesthetic toxicity has been explained by their effects on the cardiac mitochondria. In isolated mitochondria, high concentrations of bupivacaine inhibit oxidative phosphorylation, and decrease the production of adenosine triphosphate (ATP) (Cela et al., 2010; Sztark et al., 1998). The main depressive effect on the mitochondrial respiration is the inhibition of complex I (nicotinamide adenine dinucleotide [NADH] dehydrogenase) of the respiratory chain (Sztark et al., 1998).

In clinical setting of severe LAST, the seizures turn the patient rapidly hypoxaemic, hypercapnic and acidotic resulting in both respiratory and metabolic acidosis (Rosenberg et al., 1983). The acidosis exacerbates the local anaesthetic toxicity (Porter et al., 2000), as the free drug concentration rises (Burney et al., 1978) and the drug molecules are trapped inside cardiomyocytes in their ionised form (Tucker and Mather, 1975). The same exacerbation of toxicity can be seen also after hypoxaemia (Heavner et al., 1992).

**TRICYCLIC ANTIDEPRESSANTS**

TCAs were introduced for the treatment of depression in the late 1950s, imipramine
being the first one (Azima, 1959). Later, newer TCAs, such as amitriptyline (Figure 6) and nortriptyline, have mainly replaced imipramine in clinical practise. In addition to depression, the indications for TCA prescription have extended to involve not only several psychiatric disorders but also, for instance, pain syndromes, fibromyalgia and insomnia (Woolf et al., 2007). Nowadays, TCAs are not, however, considered as a first line treatment for depression anymore (Depression: Current Care Guideline 2014). In chronic pain syndromes (e.g., neuropathic pain and fibromyalgia), instead, for example amitriptyline has still an important role (Finnerup et al., 2005; Moore et al., 2012).

According to the sales numbers in Finland in 2013 (Finnish Medicines Agency, 2013), approximately 4 persons out of 1000 used some TCA, amitriptyline being the most used, at its average maintenance dose (defined daily dose [DDD] of 4.26 per 1000 persons). During the last years, the consumption rate of TCAs has been rather stable. In addition to amitriptyline being the most used TCA, it has been one of most common cause of fatal poisonings in Finland for the last decade (Vuori et al., 2009; 2012).

**Pharmacology**

TCAs are tertiary amines with two methyl groups attached to a nitrogen atom. They are lipophilic, and therefore, rapidly absorbed from the gastrointestinal tract, after which they are also quite rapidly demethylated to the active secondary amines. From the bloodstream they are distributed into tissues (volumes of distribution vary from approximately 10 to 50 l kg⁻¹ between different TCAs). The physicochemical and some pharmacokinetic properties of TCAs are presented in more detail in Table 3.

![Figure 6. Chemical structure of amitriptyline.](image)

**Table 3. Physicochemical and some pharmacokinetic properties of tricyclic antidepressants**

<table>
<thead>
<tr>
<th></th>
<th>pKₐ, at 25°C</th>
<th>LogD</th>
<th>LogP</th>
<th>Vₐ (l kg⁻¹)</th>
<th>Plasma protein binding (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline</td>
<td>9.4</td>
<td>2.96</td>
<td>4.92</td>
<td>8.9</td>
<td>95</td>
</tr>
<tr>
<td>Nortriptyline</td>
<td>10.1</td>
<td>2.28</td>
<td>5.65</td>
<td>19.1–34.6</td>
<td>92.5</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>9.3</td>
<td>3.31</td>
<td>5.39</td>
<td>11.9</td>
<td>97.25</td>
</tr>
<tr>
<td>Doxepin</td>
<td>9.0</td>
<td>2.50</td>
<td>3.86</td>
<td>89.6</td>
<td>79.6</td>
</tr>
<tr>
<td>Imipramine</td>
<td>9.5</td>
<td>2.68</td>
<td>4.80</td>
<td>11.5–17.5</td>
<td>85</td>
</tr>
</tbody>
</table>

pKₐ, dissociation constant (Embil and Torosian, 1982; Shalaeva et al., 2008); LogD, logarithm of predicted octanol:water partition coefficient at physiological pH (ChemSpider, 2015); logP, logarithm of predicted octanol:water partition coefficient at pH 7 (ChemSpider, 2015); Vₐ, volume of distribution (Scoggins et al., 1980); plasma protein binding (Ferner, 2008; Virtanen et al., 1982).
The primary antidepressive mechanism of action of TCAs is the prevention of noradrenaline, but also serotonin, uptake at nerve terminals increasing their concentration in the synaptic cleft (Ballinger and Feely, 1983; Tatsumi et al., 1997). TCAs have, however, several other effects that may be unwanted, such as anticholinergic effects (El-Fakahany and Richelson, 1983), or blockade of α1-adrenoceptors (U'Prichard et al., 1978), histamine H1 receptors (Richelson, 1978), or γ-aminobutyric acid receptors (Malatynska et al., 1988). In addition to these receptors, they also block neural sodium and calcium channels (Ishii and Sumi, 1992; Lavoie et al., 1990), likely by binding to the same receptor site as local anaesthetics (Nau et al., 2000).

As mentioned above, the enteral absorption of TCAs is marked, in some cases even better than parenteral absorption after, for example, intramuscular administration (Scoggins et al., 1980). After absorption, they undergo first-pass metabolism by cytochrome P450 (CYP) isoenzymes (mainly by CYP2D6 and CYP2C19) which determines the availability of the active metabolites, e.g. E-10-OH-nortriptyline, the active metabolite of both amitriptyline and nortriptyline (Rudorfer and Potter, 1999). The metabolism of TCAs is also dependent on the huge polymorphism of CYP2D6 (Zhou, 2009) but also that of CYP2C19 (Desta et al., 2002), which affect the drug responses. In the bloodstream, the TCAs are bound to plasma proteins, such as albumin and lipoproteins but also to α1-acid glycoprotein (Routledge, 1986), to a great extent, and hence the fraction of free drug is usually less than a quarter of the total concentration (Molnar and Gupta, 1980; Scoggins et al., 1980). In addition to marked protein binding, TCAs distribute into tissues, in which the concentration may be much higher than in blood, and as lipophilic drugs they easily cross the blood-brain barrier (Scoggins et al., 1980).

TCAs are demethylated and metabolised very efficiently mainly into hydroxy compounds through hydroxylation followed by glucuronide coupling in the liver (Scoggins et al., 1980). These metabolites are then secreted into urine (Molnar and Gupta, 1980).

**Tricyclic antidepressant poisoning**

TCAs are used as enteral drugs so the TCA poisoning is a consequence of either intentional or unintentional ingestion of large dose of a TCA alone or along with other medication or alcohol. An overdose of TCAs markedly changes their pharmacokinetics. The delaying of gastric emptying may delay absorption from gastrointestinal tract, the final elimination is delayed due to notable enterohepatic recirculation and saturation of metabolising enzymes, and the free fraction of the drug may increase due to acidosis which is a result of respiratory depression (Jarvis, 1991).

The first cardiovascular effect of TCA poisoning is sinus tachycardia due to the reduced noradrenaline uptake from synaptic clefts and anticholinergic effects (Kerr et al., 2001; Taylor and Braithwaite,
In a more serious poisoning, they delay the depolarisation in myocardium and conduction system by inhibiting sodium channels (Brennan, 1980). The inhibition of cell depolarisation can be seen as prolongation of ECG conduction times, such as widening of QRS complex and lengthening of QT interval (Liebelt et al., 1997). These ECG abnormalities also predict cardiac arrhythmias and seizures, and usually persist regardless of patient’s clinical improvement (Liebelt et al., 1997). The patient’s condition can also deteriorate later after apparent stabilisation (Levine, Brooks, et al., 2012). Similarly, the inhibition of sodium current can result in a depressed contractility in the heart (Kerr et al., 2001; Taylor and Braithwaite, 1978). Together with the cardiac effects, the blockade of peripheral α1-adrenoceptors leads to vasodilation, and hypotension (Kerr et al., 2001; U’Prichard et al., 1978).

In addition to cardiovascular effects, TCA poisoning may provoke seizures (Taboulet et al., 1995). The lowered seizure activity in serious poisonings is considered to stem from the antagonism of inhibitory γ-aminobutyric acid receptors in the CNS (Malatynska et al., 1988). Both CNS and cardiac toxicity, however, is rather difficult to predict from the ingested dose due to altered pharmacokinetics (Jarvis, 1991).

The anticholinergic effects are common, even at non-toxic doses, and may cause, for instance, ileus but rarely are serious. There is, however, a possibility of even fatal complications, such as toxic megacolon and perforation (Ross et al., 1998).

**Intravenous Lipid Emulsion**

Commercial lipid emulsions were originally developed in the 1960s to be used as a component of parenteral nutrition (MacFie, 1999). The lipids are presented as dispersed particles comparable in the size of chylomicrons (200–400 nm). The compositions of Intralipid®, one of the most commonly used lipid emulsion, is presented in Table 4. Critically ill patients, for example, in intensive care units require essential fatty acids that are the primary component of lipid emulsions (Calder et al., 2010). The lipid emulsions are composed of triglyceride-rich particles which are

| Table 4. Compositions of 20% Intralipid® (Fresenius Kabi AB, 2013; Skeie et al., 1988; Washington et al., 1993) |
|---|---|
| **Soya bean oil** | 200 mg l⁻¹ |
| Linoleic acid (C18:2) | 44–62% |
| Oleic acid (C18:1) | 19–30% |
| Palmitic acid (C16:0) | 7–14% |
| Linolenic acid (C18:3) | 4–11% |
| Stearic acid (C18:0) | 1.4–5.5% |
| Arachidinic acid (C20:4) | ∼0.6% |
| Egg yolk phosphatides | 12 mg l⁻¹ |
| Glycerin | 22.5 mg l⁻¹ |
| Particle size | 346±18 nm |
| pH | 8 (range 6 to 8.9) |
| Osmolality | 350 mOsmol kg⁻¹ (260 mOsmol l⁻¹) |
| Total caloric value | 2.0 kcal ml⁻¹ |
stabilised by phospholipids (Carpentier and Dupont, 2000). The triglycerides are typically vegetable oil based, or fish oil based in newer compounds, with a typical concentration of 10 to 30% (Carpentier and Dupont, 2000).

The fatty acids, derived from lipid emulsions, do not function only as nutrients but they also have an important role on immune modulation as they affect cell membrane fluidity, production of bioactive mediators and cell signalling (Wanten and Calder, 2007). Even gene expression and increment in lymphocyte apoptosis are modulated by fatty acids (Wanten and Calder, 2007). Even gene expression and increment in lymphocyte apoptosis are modulated by fatty acids (Wanten and Calder, 2007). Infusion of especially soya bean-based lipid emulsions seem to associate also with high blood levels of ω-6 polyunsaturated fatty acids and arachidonic acid which may lead to production of proinflammatory, but also vasoconstrictive, prostaglandins and thromboxanes (Calder et al., 2010).

Lipid emulsions are not utilised solely as nutritional solutions. As some drugs are not water-soluble but are desired to be administered i.v., lipid emulsion offers a suitable vehicle for such administration (Tamilvanan, 2004). Commercially available drugs dissolved in lipid emulsion include, for instance, propofol, diazepam, etomidate and dexamethasone (Tamilvanan, 2004).

**LIPID RESCUE**

Treatment of severe intoxications with intravenous lipid emulsion, or lipid rescue, has been developed mainly during the last 15 years. The basis of lipid rescue has been pharmacokinetic interactions between lipid emulsion and lipophilic drugs, and therefore, alleviation of toxicity symptoms. At the moment it is a recommended treatment of LAST, for instance, in the United Kingdom (UK) and the US (Cave et al., 2010; Neal et al., 2012). In the international literature, lipid rescue has also been proposed as a potential treatment of several other drug poisonings (Cave and Harvey, 2014).

**Background**

The first study of interactions between lipid emulsion and lipophilic drug, thiopental, was published soon after the introduction of the first commercially available lipid emulsion (MacFie, 1999; Russell and Westfall, 1962). Over the following decades, only few studies were published on the topic (Kriegstein et al., 1974; Minton et al., 1987; Straathof et al., 1984). Even though the idea of treating poisonings of lipophilic drugs with intravenous lipid emulsion was already invented, it did not convince the researchers at that time (Minton et al., 1987).

The birth of lipid rescue can be laid in the year 1998 when Weinberg and colleagues (1998) published the first reports suggesting that lipid rescue would actually work. They had managed to reduce bupivacaine toxicity by treating or pre-treating rats with intravenous lipid emulsion. Since then, several animal studies and human case reports have been published on lipid rescue of various drug intoxications (Bartos and Knudsen, 2013; Jamaty et al., 2010). The evidence supporting lipid rescue
Review of the literature

is, however, based almost entirely on animal studies and published case reports. After the work of Weinberg’s group, the first and only human studies, without any notable differences between the treatment with lipid emulsion and the control treatment, were published in 2012 (Litonius, Tarkkila, et al., 2012; Taftachi et al., 2012).

Mechanisms of action

Originally, the mechanism of action of lipid rescue was proposed to be the formation of a ‘lipid sink’: a lipophilic plasma phase that entraps lipophilic drug agents into itself, and therefore, prevents their action in target tissues, or even draws already distributed molecules back into the bloodstream (Weinberg et al., 1998). According to the lipid sink theory, the lipophilic drugs would sequester inside the triglyceride oil droplets in bloodstream depending on their lipophilicity (Damitz and Chauhan, 2015). This mechanism was the same that was considered ineffective a decade earlier (Minton et al., 1987) but now the lipid sink theory was considered the main mechanism of action (Neal et al., 2010; Weinberg, 2008), mainly because lipid emulsion seemed to reverse toxicity caused by various lipophilic drugs, including β-blockers (Cave et al., 2006), calcium channel blockers (Bania et al., 2007), and TCAs (Harvey and Cave, 2007). Later this theory gained more support as lipid emulsions markedly entrapped various drugs, including lipophilic local anaesthetics, in vitro (Laine et al., 2010; Lokajová et al., 2012; Mazoit et al., 2009). In vivo, however, lipid emulsion entrapped only extremely lipophilic drugs, such as amiodarone (approximately 10,000 times more lipophilic than bupivacaine) (Niiya et al., 2010) or amitriptyline (approximately 100 times more lipophilic than bupivacaine) (Litonius et al., 2012a). It seemed that local anaesthetics may not be lipophilic enough to be entrapped by lipid emulsion (Litonius et al., 2012b; Litonius, Tarkkila, et al., 2012). Very high doses of lipid emulsion, i.e., higher than those recommended in the published guidelines, can affect the distribution of bupivacaine in rats and possibly increase the metabolism by increasing the shunting into liver (Shi et al., 2013).

Lipid emulsion has been studied mainly as a treatment for cardiac toxicity, and because lipids are heart’s preferred energy substrates, another theory is that large lipid dose could reverse the inhibition of fatty acid metabolism in cardiac mitochondria (Stehr et al., 2007). This metabolism theory gained more support as Partownavid and colleagues (2012) showed that inhibition of fatty acid oxidation in cardiac mitochondria prevented also the resuscitative effect of lipid emulsion in rats. These metabolic, or other direct cardiac, effects may be even more important than the lipid sink because in more recent studies very high doses of lipid emulsion have caused a rise in arterial blood pressure, heart rate and carotid blood flow in rats, possibly through inotropic and lusitropic mechanisms (Fettiplace et al., 2013; Fettiplace, Akpa, et al., 2014).

Lipid emulsion may also have different than metabolic effects on heart. In tissue culture, free fatty acids reduce the degree of bupivacaine sodium current inhibition (Mottram et al., 2011) supporting
a theory that lipid emulsion may also affect the binding of local anaesthetics in sodium channels. In addition, cardiac calcium channels have been found to be activated by several long-chain fatty acids, e.g. oleic, linoleic and palmitic acid, at concentrations of 3 to 30 mM (Huang et al., 1992). These fatty acids are the main components of commercial lipid emulsions, such as Intralipid® (see Table 4, page 22).

It is not yet known which of the suggested mechanisms of action of lipid emulsion are the main contributors in the treatment (Harvey and Cave, 2014; Tucker, 2014). However, it seems that the original lipid sink will not explain the reversal of toxicity alone (Tucker, 2014) – at least in case of LAST.

**Lipid rescue human study in local anaesthetic systemic toxicity**

At the moment, there is only one prospective human study on the effect of lipid rescue in LAST. Litonius and colleagues (2012) showed in a randomised cross-over study that lipid emulsion infusion decreased the context-sensitive half-time ($t_{1/2}$) of total bupivacaine concentration by 44%. There were, however, no entrapment of bupivacaine, or no differences in concentrations or context-sensitive $t_{1/2}$ of free bupivacaine objecting the lipid sink theory.

**Lipid rescue animal studies in local anaesthetic systemic toxicity**

At the moment, there are at least 37 randomised controlled animal studies on the lipid rescue in LAST (Table 5). These studies include also three cell studies, and nine studies on rat aorta or heart. In addition to different models, there is a large variation in treatment, especially in dosing of lipid emulsion, and in outcome measures between the studies.

In 1998, Weinberg and colleagues (1998) published the first randomised controlled animal study on treatment of LAST with intravenous lipid emulsion. They showed that pre-treatment with different lipid emulsions increased the lethal bupivacaine dose with relation to lipid concentration. With 30% lipid emulsion, the required bupivacaine dose to produce asystole was five times higher than with saline. Later they showed that after bupivacaine-induced cardiac arrest only dogs treated with lipid emulsion in addition to resuscitation survived when compared to saline (Weinberg et al., 2003).

In rats, administration of lipid emulsion has improved the outcome in several settings. The cardiovascular outcome has improved after lipid emulsion administration both alone (Weinberg et al., 2008) and when combined with adrenaline (Hiller et al., 2009; Li et al., 2012). The combination of high adrenaline doses to lipid emulsion seemed, however, to impair the cardiovascular recovery (Hiller et al., 2009). Pre-treatment with lipid emulsion has also increased the thresholds of both convulsions and cardiac arrest (Oda and Ikeda, 2013). In resuscitation protocols, administration of very high doses of lipid emulsion has also affected the distribution of bupivacaine by, for example, decreasing the cardiac and CNS concentrations while increasing the hepatic concentration.
**Table 5. Animal studies on lipid rescue in LAST**

<table>
<thead>
<tr>
<th>Publication</th>
<th>Model</th>
<th>Local anaesthetic</th>
<th>Effect of lipid emulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weinberg et al., 1998 part 1</td>
<td>Rat</td>
<td>Bupi 75 mg kg⁻¹ min⁻¹ until cardiac arrest</td>
<td>Lethal dose was increased after lipid emulsion pre-treatment</td>
</tr>
<tr>
<td>Weinberg et al., 1998 part 2</td>
<td>Rat</td>
<td>1. Bupi 15, 17.5, 20 or 22.5 mg kg⁻¹ 2. Bupi 10, 12.5 or 15 mg kg⁻¹</td>
<td>Higher survival at similar bupivacaine dose than after saline</td>
</tr>
<tr>
<td>Weinberg et al., 2008</td>
<td>Rat</td>
<td>Bupi 20 mg kg⁻¹</td>
<td>Higher RPP and arterial oxygen pressure than after adrenaline</td>
</tr>
<tr>
<td>Hiller et al., 2009</td>
<td>Rat</td>
<td>Bupi 20 mg kg⁻¹</td>
<td>Adrenaline improved initial recovery, but after both lipid emulsion alone and combined with 1–2.5 µg kg⁻¹ adrenaline the recovery was more sustained</td>
</tr>
<tr>
<td>Li et al., 2012</td>
<td>Rat</td>
<td>Bupi 30 mg kg⁻¹</td>
<td>Haemodynamic recovery improved best after lipid emulsion and adrenaline, then after lipid emulsion alone. Cardiac bupivacaine concentration was lower after lipid emulsion and adrenaline. Adrenaline increased hypoxaemia and acidosis.</td>
</tr>
<tr>
<td>Partownavid et al., 2012</td>
<td>Rat</td>
<td>Bupi 10 mg kg⁻¹</td>
<td>Inhibition of fatty acid metabolism prevented the resuscitative effects of lipid emulsion.</td>
</tr>
<tr>
<td>Oda and Ikeda, 2013</td>
<td>Rat</td>
<td>1. Bupi 1 mg kg⁻¹ min⁻¹ 2. LBupi 1 mg kg⁻¹ min⁻¹</td>
<td>Lipid emulsion pre-treatment increased convulsive and cardiac arrest threshold</td>
</tr>
<tr>
<td>Shi et al., 2013</td>
<td>Rat</td>
<td>Bupi 2 mg kg⁻¹ min⁻¹ for 4 min</td>
<td>Bupivacaine concentration decreased in, e.g. brain and myocardium, and increased in liver. Bupivacaine half-life decreased and clearance increased.</td>
</tr>
<tr>
<td>Fettiplace, Ripper, et al., 2014</td>
<td>Rat</td>
<td>Bupi 10 mg kg⁻¹</td>
<td>Haemodynamic recovery was faster. I.v. administration of lipid emulsion was more efficacy than i.o. administration.</td>
</tr>
<tr>
<td>Fettiplace, Akpa, et al., 2014</td>
<td>Rat</td>
<td>Bupi 10 mg kg⁻¹</td>
<td>Recovery, e.g. blood pressure, RPP, and carotid flow, was faster in relation to lipid concentration.</td>
</tr>
<tr>
<td>Carreiro et al., 2014</td>
<td>Rat</td>
<td>Coca 10 mg kg⁻¹</td>
<td>Pre-treatment decreased mortality.</td>
</tr>
<tr>
<td>Fettiplace et al., 2015</td>
<td>Rat</td>
<td>Bupi 10 mg kg⁻¹</td>
<td>Haemodynamic recovery improved, bupivacaine blood concentration increased and tissue bupivacaine concentrations decreased.</td>
</tr>
<tr>
<td>Weinberg et al., 2006</td>
<td>Rat heart</td>
<td>Bupi 500 µM</td>
<td>Cardiac function recovered faster, bupivacaine concentration in heart decreased</td>
</tr>
<tr>
<td>Stehr et al., 2007</td>
<td>Rat heart</td>
<td>LBupi 5 mg l⁻¹ 1. Bupi 250 µM 2. Ropi 500 µM 3. Mepi 1000 µM</td>
<td>Systolic pressure increased</td>
</tr>
<tr>
<td>Zausig et al., 2009</td>
<td>Rat heart</td>
<td>1. Bupi 250 µM 2. Ropi 500 µM 3. Mepi 1000 µM</td>
<td>No effect on return of cardiac activity, but improved heart rate and RPP in bupivacaine intoxication</td>
</tr>
<tr>
<td>Chen et al., 2010</td>
<td>Rat heart</td>
<td>Bupi 100 µM, 40 µM 3 min after asystole</td>
<td>Recovery was improved, RPP decreased later with higher lipid concentration, myocardial bupivacaine concentration decreased</td>
</tr>
<tr>
<td>Liu et al., 2012</td>
<td>Rat heart</td>
<td>Bupi 100 µM, and 30 µM 3 min after asystole</td>
<td>Cardiac function recovered faster after lipid emulsion alone and combined with adrenaline</td>
</tr>
<tr>
<td>Chen et al., 2014</td>
<td>Rat heart</td>
<td>Bupi 40, 60, 80, 100, 120, 140 or 160 µM</td>
<td>Cardiotoxic effects were reversed in relation to bupivacaine concentration</td>
</tr>
<tr>
<td>Aumeier et al., 2014</td>
<td>Rat heart</td>
<td>1. Bupi 250 µM 2. Mepi 1000 µM</td>
<td>Time to asystole increased and recovery expedited in bupivacaine intoxication but not in mepivacaine intoxication.</td>
</tr>
<tr>
<td>Ok, Han, et al., 2013</td>
<td>Rat aorta rings</td>
<td>1. Bupi 300 µM 2. Ropi 1000 µM 3. Lido 3000 µM 4. Mepi 7000 µM</td>
<td>Lipofundin better reversed the vasodilation caused by bupivacaine compared to Intralipid. Reversal of vasodilation caused by other local anaesthetics was similar by both lipid emulsions.</td>
</tr>
<tr>
<td>Study</td>
<td>Species</td>
<td>Tissue</td>
<td>Concentration</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------</td>
<td>-------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Lee et al., 2013</td>
<td>Rat aorta rings</td>
<td>1. LBupi 600 µM 2. Ropi 2000 µM 3. Mepi 7000 µM</td>
<td>Enhanced noradrenaline-mediated vasoconstriction. The enhancement occurred at lower lipid emulsion concentration as the lipid-solubility of local anaesthetic increased.</td>
</tr>
<tr>
<td>Hori et al., 2013</td>
<td>Rat microglial cells</td>
<td>1. Bupi 1000 µM 2. Lido 1000 µM</td>
<td>Proton currents through voltage-gated proton channels improved.</td>
</tr>
<tr>
<td>Wagner et al., 2014</td>
<td>Rat cardiac myocytes</td>
<td>1. Bupi 10 µM 2. Mepi 40 µM</td>
<td>Fast Na⁺ currents were increased by 37% after lipid emulsion in bupivacaine intoxication, and slightly in mepivacaine intoxication.</td>
</tr>
<tr>
<td>Cave et al., 2009</td>
<td>Rabbit</td>
<td>Bupi 10 mg kg⁻¹</td>
<td>Survival was improved</td>
</tr>
<tr>
<td>Lemoine et al., 2014</td>
<td>Rabbit Purkinje cells</td>
<td>Bupi 1, 10 or 50 µM</td>
<td>Conduction blocks of Purkinje fibres were prevented.</td>
</tr>
<tr>
<td>Weinberg et al., 2003</td>
<td>Dog</td>
<td>Bupi 10 mg kg⁻¹</td>
<td>Recovery improved, myocardial metabolic function improved</td>
</tr>
<tr>
<td>Mayr et al., 2008</td>
<td>Pig</td>
<td>Bupi 5 mg kg⁻¹ + asphyxia</td>
<td>Adrenaline and vasopressin improved survival when compared to lipid emulsion</td>
</tr>
<tr>
<td>Hicks et al., 2009</td>
<td>Pig</td>
<td>Bupi 10 mg kg⁻¹</td>
<td>No effect on ROSC, but more noradrenaline was needed after lipid emulsion, plasma bupivacaine concentrations were higher</td>
</tr>
<tr>
<td>Candela et al., 2010</td>
<td>Pig</td>
<td>Bupi 4 mg kg⁻¹</td>
<td>Lengthening of intracardiac conduction times was reversed</td>
</tr>
<tr>
<td>Bushey et al., 2011</td>
<td>Pig</td>
<td>Bupi 5 mg kg⁻¹</td>
<td>No effect on recovery or survival</td>
</tr>
<tr>
<td>Mauch et al., 2011</td>
<td>Pig</td>
<td>Bupi 1 mg kg⁻¹ min⁻¹</td>
<td>Adrenaline improved haemodynamic recovery when compared to lipid emulsion</td>
</tr>
<tr>
<td>Mauch et al., 2012</td>
<td>Pig</td>
<td>Bupi 1 mg kg⁻¹ min⁻¹</td>
<td>Survival and haemodynamic recovery was better after adrenaline or lipid emulsion combined with adrenaline than after lipid emulsion alone</td>
</tr>
<tr>
<td>Litonius et al., 2012b</td>
<td>Pig</td>
<td>1. Bupi 2 mg kg⁻¹ min⁻¹ 2. Mepi 6 mg kg⁻¹</td>
<td>Plasma bupivacaine concentration minimally increased, no effect on haemodynamic recovery</td>
</tr>
<tr>
<td>Melo et al., 2012</td>
<td>Pig</td>
<td>Bupi 5 mg kg⁻¹ Ropi 7 mg kg⁻¹</td>
<td>Arterial blood pressure increased trough vasoconstriction</td>
</tr>
<tr>
<td>Bonfim et al., 2012</td>
<td>Pig</td>
<td>Bupi 5 mg kg⁻¹</td>
<td>Haemodynamic recovery, i.e. blood pressure, cardiac index, and peripheral resistance, improved similarly after both LCT and MCT lipid emulsions</td>
</tr>
<tr>
<td>(Litonius, Lokajová, et al., 2012)</td>
<td>Pig</td>
<td>Bupi 2 mg kg⁻¹</td>
<td>No effect on pharmacokinetics or haemodynamic recovery when Intralipid compared to POPC/POPG liposomes</td>
</tr>
<tr>
<td>de Queiroz Siqueira et al., 2014</td>
<td>Pig</td>
<td>LBupi 8.3 mg min⁻¹</td>
<td>Lipid emulsion alone and combined with adrenaline increased achieving ROSC. Adrenaline increased electrocardiogram abnormalities.</td>
</tr>
<tr>
<td>Udelsmann and Melo, 2015</td>
<td>Pig</td>
<td>Bupi 5 mg kg⁻¹</td>
<td>Haemodynamic recovery improved better after both LCT and MCT lipid emulsions.</td>
</tr>
</tbody>
</table>

Bupi, bupivacaine; Coca, cocaine; Lido, lidocaine; LBupi, levobupivacaine; LCT, long-chain triglycerides; Mepi, mepivacaine; MCT, medium-chain triglycerides; POPC/POPG, 1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine/1-palmitoyl-2-oleyl-sn-glycero-3-(phospho-rac-[1-glycerol]) dispersion; Ropi, ropivacaine; ROSC, return of spontaneous circulation; RPP, rate-pressure product.

(Fettiplace et al., 2015; Shi et al., 2013). These pharmacokinetic findings have not always been accompanied by improved cardiovascular outcome (Shi et al., 2013) even though in some studies, lipid emulsion increased blood pressure and carotid flow in bupivacaine intoxication (Fettiplace et al., 2015; Fettiplace, Akpa, et al., 2014). In bupivacaine intoxication, the accompanied inhibition of the fatty acid β-oxidation also prevents the resuscitative effect in rats suggesting a metabolic mechanism of action.
of lipid emulsion in lipid rescue (Partownavid et al., 2012).

The resuscitative effect of lipid emulsion has also been demonstrated in isolated rat heart models, as it has expedited the recovery of cardiac function and decreased bupivacaine concentrations in heart (Stehr et al., 2007; Weinberg et al., 2006). In another rat heart study, lipid emulsion improved the recovery from bupivacaine intoxication but not from ropivacaine or mepivacaine intoxication (Zausig et al., 2009). In bupivacaine intoxication, lipid emulsion has also demonstrated a dose-dependent recovery of especially heart rate (Chen et al., 2010). On the other hand, lipid emulsion has also reversed the local anaesthetic-induced vasodilation (Ok, Park, et al., 2013) and enhanced the vasoconstrictive effect of noradrenaline in rat aorta (Lee et al., 2013).

In pigs, the efficacy of lipid emulsion is more controversial. In some studies, the administration of lipid emulsion in clinically recommended or higher doses has expedited the normalisation of intracardiac conduction time prolongation (Candela et al., 2010) or improved achieving return of spontaneous circulation (de Queiroz Siqueira et al., 2014). In contrast to rats, adrenaline alone or combined with lipid emulsion has proven more effective treatment than lipid emulsion alone (Hicks et al., 2009; Mauch et al., 2011; 2012). While the internationally recommended dose of lipid emulsion has not affected the haemodynamic outcome (Litonius et al., 2012b), higher doses, as in rat studies, have improved the haemodynamic recovery (Bonfim et al., 2012; Melo et al., 2012; Udelsmann and Melo, 2015).

**Lipid rescue case reports in local anaesthetic systemic toxicity**

Due to shortage of human studies, the human experience of lipid emulsion treatment in LAST relies mainly on clinical case reports. At the moment, there are at least 42 such case report publications of lipid rescue in systemic toxicities of various local anaesthetics. The first of those were published in 2006 (Litz et al., 2006; Rosenblatt et al., 2006). The possible positive publication bias (positive results are more likely published than negative), and the heterogeneous treatment protocols and reporting accuracies between different case reports complicate the evaluation of the efficacy of lipid rescue. However, according to most of the reports, lipid rescue seems to be effective. The case reports are categorised according to the effect of lipid emulsion on the patient’s symptoms in Table 6.

In most cases, the administration of lipid emulsion seemed to abolish the various symptoms of LAST varying from mild CNS toxicity (Mizutani et al., 2011) to cardiac arrest (Scherrer et al., 2013; Sonsino and Fischler, 2009). Some authors report a dramatic effect which was seen within seconds after beginning of lipid rescue (Shenoy et al., 2014; Whiteside, 2008). On the other hand, some authors report, that despite the administration of various medications, particularly the lipid emulsion administration seemed to be the turn for the
### Table 6. Case reports on lipid rescue in LAST

<table>
<thead>
<tr>
<th>Publication</th>
<th>Local anaesthetic</th>
<th>Block</th>
<th>Symptoms at time of lipid emulsion administration</th>
<th>Suggested effect of lipid emulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipid emulsion had an immediate effect</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosenblatt et al., 2006</td>
<td>Bupi 1.2 mg kg(^{-1}), Mepi 3.7 mg kg(^{-1})</td>
<td>Interscalene</td>
<td>Cardiac arrest</td>
<td>Defibrillated to SR after lipid emulsion</td>
</tr>
<tr>
<td>Scherrer et al., 2013</td>
<td>Ropi 7.9 mg kg(^{-1})</td>
<td>TAP</td>
<td>Cardiac arrest</td>
<td>Cardiac activity immediately</td>
</tr>
<tr>
<td>Sonsino and Fischler, 2009</td>
<td>Ropi 150 mg</td>
<td>Infraclavicular</td>
<td>Cardiac arrest</td>
<td>SR immediately</td>
</tr>
<tr>
<td>Cordell et al., 2010</td>
<td>Bupi 75 mg</td>
<td>Axillary</td>
<td>VT</td>
<td>SR after lipid bolus</td>
</tr>
<tr>
<td>Shenoy et al., 2014</td>
<td>Bupi 2.3 mg kg(^{-1})</td>
<td>Caudal</td>
<td>VT</td>
<td>Sinus tachycardia immediately, SR later</td>
</tr>
<tr>
<td>Whiteside, 2008</td>
<td>Bupi 0.29 mg kg(^{-1})</td>
<td>Sciatic nerve</td>
<td>Seizure</td>
<td>Seizures stopped within seconds</td>
</tr>
<tr>
<td>Spence, 2007</td>
<td>Lido 0.9 mg kg(^{-1}), Bupi 0.17 mg kg(^{-1})</td>
<td>Epidural</td>
<td>Unresponsive</td>
<td>Consciousness within 30 s</td>
</tr>
<tr>
<td>Espinet and Emmerton, 2009</td>
<td>Bupi 1.25 mg kg(^{-1}), Lido 1.25 mg kg(^{-1})</td>
<td>Lower leg nerve</td>
<td>Mild CNS symptoms, ST segment elevation</td>
<td>Symptoms improved within minute</td>
</tr>
<tr>
<td>Charbonneau et al., 2009</td>
<td>Mepi 14.9 mg kg(^{-1})</td>
<td>Axillary</td>
<td>Myoclonus</td>
<td>Symptoms disappeared immediately</td>
</tr>
<tr>
<td><strong>The effect of lipid emulsion was seen within minutes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith et al., 2008</td>
<td>Bupi 1.7 mg kg(^{-1})</td>
<td>Sciatic nerve</td>
<td>Cardiac arrest</td>
<td>SR after 9 min</td>
</tr>
<tr>
<td>Hurley and Hanlon, 2009</td>
<td>Bupi</td>
<td>–</td>
<td>Cardiac arrest</td>
<td>Clinical improvement within minutes</td>
</tr>
<tr>
<td>Marwick et al., 2009</td>
<td>Bupi 1.6 mg kg(^{-1})</td>
<td>Infraclavicular</td>
<td>Cardiac arrest</td>
<td>Narrow complexes within 3 min, but periods of VT 40 min later. Also pancreatitis</td>
</tr>
<tr>
<td>Greniec et al., 2011</td>
<td>Lido 20 mg</td>
<td>Epidural</td>
<td>Cardiac arrest</td>
<td>SR within 4 min</td>
</tr>
<tr>
<td>Markowitz and Neal, 2009</td>
<td>Bupi 1.6 mg kg(^{-1})</td>
<td>Femoral nerve</td>
<td>VF</td>
<td>Defibrillated to SR within minutes</td>
</tr>
<tr>
<td>Gallagher et al., 2010</td>
<td>Bupi 1.9 mg kg(^{-1}), Lido 10.2 mg kg(^{-1})</td>
<td>Subpectorial region</td>
<td>PEA</td>
<td>Pulse restored during second lipid dose</td>
</tr>
<tr>
<td>Dix et al., 2011</td>
<td>Lido blood concentration 7.6 mg l(^{-1})</td>
<td>I.v. infusion</td>
<td>PEA</td>
<td>SR after 5 min</td>
</tr>
<tr>
<td>Foxall et al., 2007</td>
<td>LBupi 1.2 mg kg(^{-1})</td>
<td>Posterior lumbar plexus</td>
<td>Seizures and CV collapse</td>
<td>BP normalisation after 5 min</td>
</tr>
<tr>
<td>McCutchen and Gerancher, 2008</td>
<td>Bupi 150 mg, Ropi 150 mg</td>
<td>Femoral nerve</td>
<td>VT</td>
<td>Defibrillated to SR some minutes after lipid emulsion</td>
</tr>
<tr>
<td>Ludot et al., 2008</td>
<td>Lido 1.8 mg kg(^{-1}), Ropi 1.4 mg kg(^{-1})</td>
<td>Posterior lumbar plexus</td>
<td>VT</td>
<td>SR after 2 min</td>
</tr>
<tr>
<td>Fuzaylov et al., 2010</td>
<td>Bupi 0.5 mg kg(^{-1})</td>
<td>I.v. bolus</td>
<td>VT</td>
<td>Sinus tachycardia within 2 min, but hypotension persisted</td>
</tr>
<tr>
<td>Arora et al., 2013</td>
<td>Coca</td>
<td>Smoking of crack coca</td>
<td>Wide complex tachycardia, seizures</td>
<td>SR within 10 min</td>
</tr>
<tr>
<td>Lin and Aronson, 2010</td>
<td>Bupi 1 mg kg(^{-1})</td>
<td>Caudal epidural</td>
<td>Ventricular complexes</td>
<td>SR within 3 min</td>
</tr>
</tbody>
</table>
### Review of the literature

<table>
<thead>
<tr>
<th>Study</th>
<th>Anesthesia</th>
<th>Site</th>
<th>Outcome</th>
<th>Time Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litz et al., 2008</td>
<td>Mepi 5.3 mg kg(^{-1}), Prilo 1.75 mg kg(^{-1})</td>
<td>Infraclavicular</td>
<td>Unresponsive</td>
<td>Consciousness after 5 min</td>
</tr>
<tr>
<td>Shah et al., 2009</td>
<td>Bupi 2.0 mg kg(^{-1})</td>
<td>Caudal epidural</td>
<td>ST segment elevation</td>
<td>ECG normalisation within minutes</td>
</tr>
<tr>
<td>Bilotta et al., 2012</td>
<td>Lido 500 mg, Ropi 300 mg kg(^{-1})</td>
<td>Subcutaneously in head</td>
<td>Complete atioventricular block</td>
<td>The block resolved rapidly</td>
</tr>
<tr>
<td>Shih et al., 2011</td>
<td>Bupi 0.8 mg kg(^{-1}), Lido 4.6 mg kg(^{-1})</td>
<td>Infraclavicular</td>
<td>Junctional bradycardia</td>
<td>SR within 2 min</td>
</tr>
<tr>
<td>Wong et al., 2010</td>
<td>Bupi 3.1 mg kg(^{-1})</td>
<td>Epidural</td>
<td>Sinus tachycardia and hypotension</td>
<td>BP increased</td>
</tr>
<tr>
<td>Diaz et al., 2012</td>
<td>Bupi 0.5 mg kg(^{-1}), Lido 4.5 mg kg(^{-1})</td>
<td>Epidural</td>
<td>Unconsciousness, hypotension</td>
<td>Consciousness within 3 min</td>
</tr>
<tr>
<td>Mizutani et al., 2011</td>
<td>Ropi 3.0 mg kg(^{-1})</td>
<td>Interscalene</td>
<td>Incoherence and other mild CNS symptoms</td>
<td>Symptoms disappeared within minutes</td>
</tr>
<tr>
<td>Nguyen and White, 2012</td>
<td>Ropi 1.0 mg kg(^{-1})</td>
<td>Supraclavicular</td>
<td>Disorientation</td>
<td>Symptoms disappeared within 2 min</td>
</tr>
</tbody>
</table>

**The effect of lipid emulsion was seen gradually**

<table>
<thead>
<tr>
<th>Study</th>
<th>Anesthesia</th>
<th>Site</th>
<th>Outcome</th>
<th>Time Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litz et al., 2006</td>
<td>Ropi 8 mg kg(^{-1})</td>
<td>Axillary</td>
<td>Cardiac arrest</td>
<td>ROSC after 10 min</td>
</tr>
<tr>
<td>Whitman and Kushins, 2014</td>
<td>Bupi 13 mg kg(^{-1})</td>
<td>Abdominal intramuscular pain</td>
<td>Cardiac arrest</td>
<td>ROSC after 45 min</td>
</tr>
<tr>
<td>Warren et al., 2008</td>
<td>Mepi 5.4 mg kg(^{-1}), Bupi 0.6 mg kg(^{-1})</td>
<td>Supraclavicular</td>
<td>VF</td>
<td>ROSC after 11 min</td>
</tr>
<tr>
<td>Varela and Bums, 2010</td>
<td>Bupi 2.1 mg kg(^{-1}), Ropi 4.3 mg kg(^{-1})</td>
<td>Femoral and sciatic nerve</td>
<td>VT</td>
<td>SR after a 30-min lipid infusion</td>
</tr>
<tr>
<td>Jakkala-Saibaba et al., 2011</td>
<td>Coca</td>
<td>Nasal inhalation</td>
<td>Seizures and VT periods</td>
<td>SR after 15 min</td>
</tr>
<tr>
<td>Harvey et al., 2011</td>
<td>Bupi 1.9 mg kg(^{-1}), Lido 0.6 mg kg(^{-1})</td>
<td>Femoral nerve</td>
<td>Seizures and hypotension</td>
<td>BP normalisation within 20 min</td>
</tr>
<tr>
<td>Lange et al., 2012</td>
<td>Lido 26 mg kg(^{-1})</td>
<td>Abdominal intramuscular injection</td>
<td>Lowered level of consciousness</td>
<td>Oriented but still lethargic after 10 min</td>
</tr>
</tbody>
</table>

**No effect or time scale not reported**

<table>
<thead>
<tr>
<th>Study</th>
<th>Anesthesia</th>
<th>Site</th>
<th>Outcome</th>
<th>Time Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gnaho et al., 2009</td>
<td>Ropi 2.2 mg kg(^{-1})</td>
<td>Sciatic nerve</td>
<td>VF (worst symptom)</td>
<td>No more symptoms at time of lipid administration</td>
</tr>
<tr>
<td>Kundu et al., 2013</td>
<td>Coca</td>
<td>Axillary</td>
<td>Seizures, wide complex arrhythmias</td>
<td>No effect</td>
</tr>
<tr>
<td>Calenda and Dinescu, 2009</td>
<td>Ropi 1.9 mg kg(^{-1}), Mepi 5.0 mg kg(^{-1})</td>
<td>Axillary</td>
<td>Seizures</td>
<td>No effect</td>
</tr>
<tr>
<td>Aveline et al., 2010</td>
<td>Lido 7 mg kg(^{-1}), Ropi 2.0 mg kg(^{-1})</td>
<td>Sciatic nerve</td>
<td>Seizures</td>
<td>No effect</td>
</tr>
<tr>
<td>Landy et al., 2012</td>
<td>Lido 5 mg kg(^{-1})</td>
<td>Brachial plexus</td>
<td>Seizures</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

---

Bupi, bupivacaine; BP, blood pressure; Coca, cocaine; CNS, central nervous system; CV, cardiovascular; ECG, electrocardiogram; Lido, lidocaine; LBupi, levobupivacaine; Mepi, mepivacaine; Prilo, prilocaine; PEA, pulseless electrical activity; Ropi, ropivacaine; ROSC, return of spontaneous circulation; SR, sinus rhythm; TAP, transversus abdominis plane; VF, ventricular fibrillation; VT, ventricular tachycardia.
better in patient’s condition (Gallagher et al., 2010; Litz et al., 2006; Rosenblatt et al., 2006; Shah et al., 2009; Shih et al., 2011; Wong et al., 2010). There are, however, also two cases in which lipid emulsion did not influence the symptoms of LAST as the seizure activity (Aveline et al., 2010; Calenda and Dinescu, 2009) and a third in which the seizure activity accompanied with ventricular arrhythmias continued (Kundu et al., 2013) despite lipid rescue. However, the two former patients survived without any sequelae.

Despite convincing case reports, the connection of lipid emulsion administration to the recovery of the patient can be questioned in many cases. In some cases, for example, lipid emulsion had been given just prior to first defibrillation (Markowitz and Neal, 2009), even with other medications, such as amiodarone, (McCutch en and Gerancher, 2008), and as the rhythm successfully converted to sinus rhythm, the benefit of lipid emulsion remains uncertain. Similarly, some patients were administered several medications during the cardiopulmonary resuscitation and the effect of lipid emulsion cannot with certainty be associated with the improvement of patient’s clinical status (Cordell et al., 2010; Grec et al., 2011; Smith et al., 2008; Warren et al., 2008).

Lipid emulsion has been administered at the same time with other medication, e.g. midazolam, to treat minor CNS intoxication (Nguyen and White, 2012), and also rather mild CNS pretoxicity symptoms, such as incoherence, slurred speech (Mizutani et al., 2011). Such symptoms are typically short-lasting (Haasio et al., 1988) and may probably have passed rapidly even spontaneously.

There are also some alarming characteristics that can be noted in some case reports. Many anaesthesiologists seem to consider lipid emulsion as the first line treatment for LAST (Lange et al., 2012; Mizutani et al., 2011; Scherrer et al., 2013). In one of the most recent case, the authors reported no use of other medications than lipid emulsion despite that the cardiopulmonary resuscitation lasted for 45 min and required two defibrillations before return of spontaneous circulation (Whiteman and Kushins, 2014).

In addition to modern local anaesthetics, lipid emulsion has been used to treat cocaine overdoses (Arora et al., 2013; Jakkala-Saibaba et al., 2011; Kundu et al., 2013). The patients were given various other medications prior to lipid emulsion, but the latter seemed to improve the patients’ status markedly in two cases (Arora et al., 2013; Jakkala-Saibaba et al., 2011). On the other hand, in one of the three patients lipid emulsion had no effect on the wide complex ECG arrhythmias or the seizure activity even though the lipid treatment was given repeatedly (Kundu et al., 2013). In some of the case reports, lipid rescue itself seemed to cause complications. These events are presented below in a separate paragraph ‘Adverse effects’.

Lipid rescue human studies in other drug poisonings
There are currently only two prospective human studies of lipid rescue in other drug poisonings than those caused by local anaesthetics. Minton and colleagues (1987)
showed in a randomised cross-over study that infusion of 500 ml of 20% lipid emulsion during 5 h increased only minimally total amitriptyline and nortriptyline concentrations after amitriptyline administration but there was no statistically significant difference between lipid emulsion and saline treatments. In the other study, lipid emulsion treatment showed no clinically significant changes in patient population of heterogeneous drug poisonings (Taftachi et al., 2012).

**Lipid rescue animal studies in tricyclic antidepressant poisonings**

The use of lipid emulsion in treatment of TCA poisoning is much less investigated as in treatment of LAST. At the moment, there are six controlled animal studies on the subject (Table 7). As TCAs are very lipophilic, lipid emulsion has entrapped both amitriptyline and clomipramine in plasma (Harvey et al., 2009; Litonius et al., 2012a; Perichon et al., 2013). The haemodynamic outcome has, however, varied greatly between different studies. In rats and rabbits, after administration of amitriptyline or clomipramine i.v., lipid emulsion has been a superior treatment over both saline and sodium bicarbonate (Bania and Chu, 2006; Harvey and Cave, 2007). Also intraperitoneal lipid emulsion dialysis, combined with an i.v. therapy, has proven beneficial (Harvey et al., 2009).

After oral amitriptyline ingestion in rats, lipid emulsion has, however, increased mortality and retarded recovery (Perichon et al., 2013). Similar negative cardiovascular effect (Varney et al., 2014) or no effect at all (Litonius et al., 2012a) of lipid emulsion was seen in pigs after an i.v. administration of amitriptyline.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Model</th>
<th>Tricyclic antidepressant</th>
<th>Effect of lipid emulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bania and Chu, 2006</td>
<td>Rat</td>
<td>Amitriptyline 42 mg kg⁻¹ in 1 h i.v.</td>
<td>After lipid emulsion pre-treatment, MAP was higher at 45 min, higher mortality, higher amitriptyline plasma concentrations, lower BP than after sodium bicarbonate treatment</td>
</tr>
<tr>
<td>Perichon et al., 2013</td>
<td>Rat</td>
<td>Amitriptyline 70 mg kg⁻¹ p.o.</td>
<td>Higher mortality, higher amitriptyline plasma concentrations, lower BP than after sodium bicarbonate</td>
</tr>
<tr>
<td>Harvey and Cave, 2007</td>
<td>Rabbit</td>
<td>Clomipramine 320 mg kg⁻¹ h⁻¹ i.v. until MAP 50%</td>
<td>MAP recovered faster than after sodium bicarbonate</td>
</tr>
<tr>
<td>Harvey and Cave, 2007 part 2</td>
<td>Rabbit</td>
<td>Clomipramine 240 mg kg⁻¹ h⁻¹ i.v. until MAP 25 mmHg</td>
<td>Survival was better than after sodium bicarbonate</td>
</tr>
<tr>
<td>Harvey et al., 2009</td>
<td>Rabbit</td>
<td>Clomipramine 240 mg h⁻¹ i.v. until MAP 50%</td>
<td>After i.v. and intraperitoneal lipid emulsion treatment, MAP recovered faster, higher clomipramine concentration in plasma than after saline</td>
</tr>
<tr>
<td>Litonius et al., 2012a</td>
<td>Pig</td>
<td>Amitriptyline 15 mg kg⁻¹ i.v. in 15 min</td>
<td>Higher amitriptyline concentration in plasma than after Ringer’s acetate, no haemodynamic effect</td>
</tr>
<tr>
<td>Varney et al., 2014</td>
<td>Pig</td>
<td>Clomipramine 0.5 mg kg⁻¹ min⁻¹ i.v. until MAP 60%, then infusion of 10% of the initial dose</td>
<td>Higher mortality, lower BP than after sodium bicarbonate treatment</td>
</tr>
</tbody>
</table>

BP, blood pressure; MAP, mean arterial pressure.
Lipid rescue case reports in tricyclic antidepressant poisonings

At the moment, there are at least 17 different case reports of administration of lipid emulsion as a treatment for TCA poisoning (Table 8). All patients have been treated with several other medications so the effect of lipid emulsion may be difficult to interpret. The cases can be, however, divided to three different groups. In the first, the patient’s condition, especially blood pressure and cardiac conduction times, seemed to improve almost immediately after lipid emulsion administration (Agarwala et al., 2014; Blaber et al., 2012; Boegevig et al., 2011; Engels and Davidow, 2010; Harvey and Cave, 2012; Levine, Brooks, et al., 2012; Scholten et al., 2012). In some other patients, the improvement was seen more gradually within an hour or longer time (Al-Duaij et al., 2009; Carr et al., 2009; Eren Cevik et al., 2014). Only one patient seemed not to benefit from lipid emulsion at all (Kiberd and Minor, 2012).

The adverse effects of lipid emulsion, reported also in some of these case reports, are presented below in a separate paragraph ‘Adverse effects’.

Adverse effects of lipid emulsions

There are no studies regarding the potential adverse effects of high lipid emulsion doses used in lipid rescue. In their original use, parenteral nutrition, they are, however, considered safe (Waitzberg et al., 2006), but also the doses are much lower. Using the recommended rescue doses, Litonius and colleagues (2012) are the only who have controlledly documented the lack of severe adverse effects in healthy human volunteers.

According to various lipid rescue case reports, the administration of high lipid emulsion dose may be associated with several distinct adverse effects. One of the most often reported complication is pancreatitis (Bucklin et al., 2013; Levine et al., 2014; Levine, Brooks, et al., 2012; Levine, Graeme, et al., 2012; Marwick et al., 2009; Oakes et al., 2009). Another often reported adverse effect is acute respiratory distress syndrome (ARDS) (Levine et al., 2014; Levine, Brooks, et al., 2012; Martin et al., 2014). In addition to organ specific complications, rapid lipid emulsion administration can result in fat overload syndrome which include several symptoms, e.g., anaemia, leukopenia, thrombocytopenia, jaundice, hepatosplenomegaly, respiratory distress, and spontaneous haemorrhages (Hojsak and Kolaček, 2014).

In parenteral nutrition, intravenous lipid emulsions have been associated with both acute hypersensitivity reactions (Lunn and Fausnight, 2011; Weidmann et al., 1997), even anaphylaxis (Ghatak et al., 2014), and other complications after prolonged infusions. These complications include, for example, immunologic effects by impairing neutrophil function (Versleijen et al., 2010), and increasing inflammatory markers while decreasing endothelial function (Umpierrez et al., 2009). The effects on vein endothelium may also cause increased risk of vein thrombosis (Smirniotis et al., 1999). A case of deep venous thrombosis has been recently reported also after lipid rescue treatment (Schwarz et al., 2014). At least long-term infusion of lipid emulsions may also cause
Table 8. Case reports on lipid rescue in tricyclic antidepressant poisonings

<table>
<thead>
<tr>
<th>Publication</th>
<th>Tricyclic antidepressant p.o.</th>
<th>Symptoms at time of lipid emulsion administration</th>
<th>Suggested effect of lipid emulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive effect or possibly positive effect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-Duaij et al., 2009</td>
<td>Imipramine 6 g</td>
<td>Unresponsive, hypotensive, arrhythmias</td>
<td>Heart rate stabilised, arrhythmias less frequent</td>
</tr>
<tr>
<td>Cooper et al., 2010</td>
<td>Amitriptyline 350 mg and several other drugs</td>
<td>Hypotensive, complete heart block</td>
<td>SR and BP improved</td>
</tr>
<tr>
<td>Boegevig et al., 2011</td>
<td>Dosulepin 5.25 g</td>
<td>Wide QRS complex, unconscious</td>
<td>QRS shortened within 15 min</td>
</tr>
<tr>
<td>Hendron et al., 2011</td>
<td>Dosulepin 45 mg kg⁻¹</td>
<td>Disoriented, VT</td>
<td>Sinus tachycardia after defibrillation</td>
</tr>
<tr>
<td>Levine, Brooks, et al., 2012</td>
<td>Amitriptyline</td>
<td>Seizures, wide-complex tachycardia</td>
<td>Seizures ceased, sinus tachycardia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Also pancreatitis and ARDS</td>
</tr>
<tr>
<td>Levine, Graeme, et al., 2012</td>
<td>Doxepin</td>
<td>Brady-asystolic arrest</td>
<td>Heart rate increased. Also pancreatitis</td>
</tr>
<tr>
<td>Blaber et al., 2012</td>
<td>Dosulepin 2.25 g</td>
<td>Wide-complex tachycardia</td>
<td>Immediate narrowing of QRS</td>
</tr>
<tr>
<td>Scholten et al., 2012</td>
<td>Amitriptyline and other drugs</td>
<td>Wide QRS complex</td>
<td>BP increased, QRS narrowed within 2 h, seizures continued</td>
</tr>
<tr>
<td>Perza et al., 2013</td>
<td>Doxepin and other drugs</td>
<td>Seizures, hypotensive, wide QRS complex</td>
<td></td>
</tr>
<tr>
<td>Nair et al., 2013</td>
<td>Amitriptyline 5.6 g, citalopram</td>
<td>GCS 3, VT runs</td>
<td>No arrhythmias after lipid</td>
</tr>
<tr>
<td>Bowler and Nethercott, 2014</td>
<td>Amitriptyline and liraglutide</td>
<td>Hypotensive, wide QRS complex</td>
<td>QRS normalised and BP increased</td>
</tr>
<tr>
<td>Agarwala et al., 2014</td>
<td>Amitriptyline 2.25 g</td>
<td>Hypotensive, wide QRS complex</td>
<td>QRS narrowed within minutes</td>
</tr>
<tr>
<td>Carr et al., 2009</td>
<td>Doxepin 19 mg kg⁻¹</td>
<td>GCS 3, hypotensive</td>
<td>BP stabilisation 2.5 h later</td>
</tr>
<tr>
<td>Engels and Davidow, 2010</td>
<td>Amitriptyline 4.25 g</td>
<td>Hypotensive</td>
<td>Noradrenaline need rapidly decreased</td>
</tr>
<tr>
<td>Harvey and Cave, 2012</td>
<td>Amitriptyline 43 mg kg⁻¹ and several other drugs</td>
<td>Hypotensive, tachycardic</td>
<td>BP begun to normalise immediately</td>
</tr>
<tr>
<td>Eren Cevik et al., 2014</td>
<td>Amitriptyline 925 mg</td>
<td>GCS 10, hypotensive</td>
<td>GCS 14 and BP increased within 1 h</td>
</tr>
<tr>
<td>Eren Cevik et al., 2014</td>
<td>Amitriptyline 875 mg</td>
<td>GCS 7, tachycardic</td>
<td>GCS 9, heart rate decreased within 1 h</td>
</tr>
<tr>
<td>Eren Cevik et al., 2014</td>
<td>Amitriptyline 520 mg</td>
<td>GCS 12, hypotensive, tachycardic</td>
<td>BP increased, heart rate decreased within 1 h</td>
</tr>
<tr>
<td>Eren Cevik et al., 2014</td>
<td>Amitriptyline</td>
<td>Hypotensive, tachycardic</td>
<td>BP increased, heart rate decreased within 20 min</td>
</tr>
<tr>
<td>Eren Cevik et al., 2014</td>
<td>Amitriptyline</td>
<td>GCS 8</td>
<td>GCS 13 within 1 h</td>
</tr>
<tr>
<td>No effect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiberd and Minor, 2012</td>
<td>Amitriptyline</td>
<td>Hypotensive, wide-complex tachycardia episodes</td>
<td>No effect on QRS duration nor on BP</td>
</tr>
</tbody>
</table>

ARDS, acute respiratory distress syndrome; BP, blood pressure; GCS, Glasgow coma scale score; SR, sinus rhythm; VT, ventricular tachycardia.

Several severe liver complications (de Meijer et al., 2009; Fallon et al., 2010).

In addition to various complications directly affecting the patient’s health, the lipaemic plasma may interfere with several laboratory tests even despite ultracentrifugation (Dimeski, 2009; Grunbaum et al., 2012; Levine et al., 2014; Watt et al., 2009). This interference may have indirect deleterious effects on the patient’s health, as some other necessary treatments may be delayed due to false or
unobtainable test results. The lipaemic plasma itself may also have serious effects as it can obstruct the filters during haemofiltration (Jeong, 2014; Rodriguez et al., 2014) and complicate extracorporeal membrane oxygenation (Lee et al., 2015). Similarly, lipid emulsions may interact, and even entrap, with other medications used in patient’s treatment (Niiya et al., 2010), which may affect their efficacy.

The present lipid emulsion dose in treatment of LAST is based on expert opinion: 1.5 ml kg$^{-1}$ in 1 min followed by an infusion of 0.25 ml kg$^{-1}$ min$^{-1}$ (Neal et al., 2010). At the moment, the recommended maximum dose varies from 10 ml kg$^{-1}$ during the first 30 min (Neal et al., 2012) to cumulative dose of 12 ml kg$^{-1}$ (Cave et al., 2010). There are, however, no studies regarding the maximum safe dose in humans.
PURPOSE OF THE STUDY
The purpose of the present study was to learn the incidence of LAST and how widely used its treatment with i.v. lipid emulsion is in Finland, and to examine different proposed mechanisms of action of i.v. lipid emulsion treatment in drug intoxication. As lipid rescue is officially recommended as a treatment for LAST in several countries but its potential mechanisms of action remain uncertain, studies on different mechanisms are highly warranted.

The specific aims were:
1. To assess the incidence of serious LAST and how widely lipid rescue is adopted in Finnish anaesthesia departments (I).
2. To evaluate if lipid emulsion can affect also milder cases of LAST: CNS toxicity caused by lidocaine and LAST which is accompanied by severe metabolic and respiratory acidosis mimicking seizures (II–III).
3. To investigate if lipid emulsion entraps local anaesthetics lidocaine, levobupivacaine and bupivacaine, and the tricyclic antidepressant amitriptyline in plasma and affects their pharmacokinetics (II–V) and haemodynamic recovery (III–V).
4. To examine if lipid emulsion in higher dose than recommended can improve cardiovascular recovery by affecting cardiac mitochondrial respiration (V).
METHODS
This thesis consists of five different studies. The Study I was made by using a structured questionnaire. The Study II is a human study with healthy volunteers while the rest of the studies (III-V) are animal studies with anaesthetised pigs.

QUESTIONNAIRE STUDY (I)
A structured electronic questionnaire using the e-form service provided by the University of Helsinki was sent via e-mail to anaesthesia department chiefs of all public hospitals in Finland in February 2014. The questionnaire is presented in detail in Appendix 1. In total, the questionnaire was sent to 5 university hospitals (comprising 18 separate university hospital-affiliated hospital units), 16 central hospitals, 10 district or local hospitals, and the Invalid Foundation Orthopaedic Hospital (n = 45). The department chiefs’ e-mail addresses were received from the register of the Finnish Society of Anaesthesiologists. If the chief did not respond, two reminders were sent, and eventually, any remaining non-respondents were contacted by telephone. Later, if LAST cases were reported, the department chief (and the anaesthesiologists involved, if necessary) was contacted again to clarify some technical issues (e.g., usage of ultrasound guidance and type of regional anaesthesia) that preceded the cases. The spinal blocks were later excluded from the study as the low local anaesthetic dose in the procedure is unlike to cause LAST.

HUMAN VOLUNTEER STUDY (II)
The study protocol was approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District (119/13/03/00/2012), and it was conducted in the laboratory of Clinical Neurophysiology at Meilahti Hospital, Helsinki University Central Hospital. Eight non-smoking Caucasian male volunteers aged 20 to 27 years and with a mean (range) body mass index of 24 (20 to 29) kg m⁻² were recruited to the study. All volunteers were determined to be healthy according to their medical history and routine laboratory tests, and none used regular medication.

The study protocol is presented in Figure 7. The study was conducted using a randomised crossover protocol. The volunteers were treated with both lipid emulsion and placebo in randomised order in two sessions which were separated by a washout period of at least two weeks.

Electroencephalography (EEG) was recorded using the Electro-Cap (Electro-Cap International Inc., Eaton, OH, USA) International 10 to 20-electrode setup (24 channels, 500 samples s⁻¹) connected to the NicOne EEG device (Natus Medical Inc., Pleasanton, CA, USA), and with simultaneous video monitoring. The volunteers were continuously monitored using a five-lead ECG, non-invasive blood pressure, pulse oximetry sensor attached to a finger, and nasal catheter capnography. Then, cubital veins in both forearms were cannulated using 17 or 16-gauge cannulas: one arm for the medication administration, and the other for blood sampling. The arm into which medications were administered
Figure 7. Study II protocol. EEG, electroencephalography.

and the infusion pumps were covered with a sheet to blind the subject and EEG analyst.

After recording baseline values, the volunteers were given a 1.5-ml kg⁻¹ i.v. bolus of treatment solution (either lipid emulsion or Ringer’s acetate) in 1 min. Then after a rapid saline flush, they were administered 1.0 mg kg⁻¹ of lidocaine i.v. followed by another saline flush. After lidocaine administration, the same treatment solution was infused 0.25 ml kg⁻¹ min⁻¹ for 30 min. The EEG technician announced the time by minute until 10 min after lidocaine administration in the otherwise quiet room. Five minutes after the lidocaine administration, the subjective severity of CNS symptoms (numbness mouth/tongue, tingling mouth/tongue, metallic taste, auditory disturbance, dizziness/lightheadedness, incoherence, sensation of twitching) was asked for separate time-points of 1, 2, 3, 4 and 5 min on a three-grade scale: no sensation (0 points), mild sensation (1 point) or strong sensation (2 points). The points were then summed separately for each symptom and volunteer at each time point.

The continuous variables were recorded and blood samples collected at baseline, and 1, 5, 10, 20 and 30 min after the lidocaine administration. A 30-s EEG epoch (eyes closed) free of artefacts was recorded 60 s before administration of lidocaine, and another 120-s EEG epoch was recorded starting 20 s after lidocaine administration.

The chosen lidocaine dose was known to be safe, but high enough to produce easily recognisable subjective toxicity symptoms (Haasio et al., 1988).

ANIMAL STUDIES (III–V)

After obtaining an approval from the National Animal Experiment Board (ESAVI-2010-08544/Ym-23, ESAVI/4536/04.10.07/2013 and ESAVI/5027/04.10.07/2014), all animal studies were conducted in the Research and Development Unit at Meilahti Hospital,
Helsinki University Central Hospital, using landrace pigs of either sex weighing 20–30 kg. The estimation of body surface area for cardiac measurements in Study V was based on the weight (Kelley et al., 1973). Before the study, the pigs were fasted over night with a free access to water. No premedication was used in any of the studies. Anaesthesia was induced with inhaled 5% isoflurane in 21% oxygen using a mask. After the induction, the pigs’ tracheas were intubated and they were mechanically ventilated with 2% isoflurane in 21% oxygen (Servo Ventilator 900C; Siemens-Elema, Solna, Sweden). The tidal volume of ventilation was adjusted to keep the end-tidal carbon dioxide (EtCO₂) concentration in the range 5.0 to 5.5% at a fixed frequency of 20 breaths min⁻¹.

Peripheral oxygen saturation (SpO₂) was monitored via a pulse oximetry sensor attached to the tail. A five-lead ECG was continuously recorded using surface electrodes.

A peripheral vein in both ears was cannulated for continuous administration of i.v. fluids. In Studies III–IV, a catheter (Leader Cath 19G; Vygon, Ecouen, France) and in Study V a two-lumen central venous catheter (7 Fr.; Arrow International, Inc., Reading, PA, USA) was inserted into one of the internal jugular veins for continuous monitoring of central venous pressure (CVP). The arterial blood pressure was monitored via cannula (Arterial Cannula with Flo-Switch 20G; Beckton-Dickinson, Singapore) inserted into the femoral artery that was exposed by dissection. In Study V, a PiCCO® Catheter (5F; Pulsion Medical Systems SE, Feldkirchen, Germany) for cardiac output and other haemodynamic measurements was inserted into the other femoral artery, and a lateral thoracotomy was performed to expose the wall of the left ventricle of the heart.

After preparations, the haemodynamics was allowed to stabilise for approximately 30 min. During this stabilisation period the oesophageal temperature was adjusted to the range 37.5 to 39.0°C using warming mattresses and an external radiant heater (OPN Ceiling Control Unit Type VII; Aragona, Sweden). CVP was kept between 2 and 8 mmHg by infusing Ringer’s acetate solution (Ringer-Acetat Baxter Viaflo®; Baxter Medical, Kista, Sweden).

All continuous variables (i.e., heart rate, mean arterial pressure [MAP], SpO₂ and EtCO₂) and ECG were recorded with a computer connected to a multimodular patient monitor (Datex-Ohmeda Division; Instrumentarium Corp, Helsinki, Finland), and running a data collection software (iCentral® and S/5 Collect®; GE Healthcare, Helsinki, Finland). These variables were later analysed at the same time points that blood samples were collected. In Study V, systemic arterial pressures, continuous cardiac output and systemic vascular resistance were monitored and recorded using a PiCCO® PulsionFlex® monitor (V4.0.0.7 A; Pulsion Medical Systems SE, Feldkirchen, Germany).

**Study III (levobupivacaine in pigs)**
The study protocol is presented in Figure 8. The pigs were paralysed using 4 mg pancuronium bromide i.v. (Pancuronium-
Methods

Figure 8. Study III protocol. FiO₂, fraction of inspired oxygen.

Actavis® 2 mg ml⁻¹; Actavis Group PTC ehf., Hafnarfjörður, Iceland) 10 min prior to the levobupivacaine administration. Then, after recording baseline values, levobupivacaine (Chirocaine® 7,5 mg ml⁻¹; AbbVie Oy, Espoo, Finland) was rapidly administered into the internal jugular vein. To produce hypercapnia, the minute ventilation was immediately reduced by 30% for 5 min. One minute after the levobupivacaine administration, 1 mmol kg⁻¹ of lactic acid (LD-Lactic acid 90% [T]; Sigma-Aldrich Co., St. Louis, MO, USA; diluted to 4 mmol ml⁻¹ with isotonic saline) was infused into the internal jugular vein to produce lactic acidosis. The pigs were then randomly treated according to the present treatment guidelines in LAST in humans: 1.5 ml kg⁻¹ in 1 min followed by 0.25 ml kg⁻¹ min⁻¹ for 29 min (Cave et al., 2010; Neal et al., 2012). After 5 minutes of hypoventilation, the minute ventilation was returned to the original level and inhaled oxygen was raised to 100% for the rest of the treatment infusion time.

Arterial blood samples were drawn and arterial blood gas analyses (ABL800 FLEX; Radiometer Medical ApS, Copenhagen, Denmark) were made from blood samples drawn as shown in Figure 8. Thirty minutes after the end of the treatment infusion, the pigs were euthanised with an i.v. bolus of potassium chloride.

Study IV (amitriptyline in pigs)
The study protocol is presented in Figure 9. After recording baseline values, the pigs were infused 10 mg kg⁻¹ of amitriptyline HCl (Sigma, Darmstadt, Germany) into the internal jugular vein at a constant infusion rate in 15 min. After a distribution period of 30 min, the pigs were randomly treated i.v. with either lipid emulsion (Intralipid® 20%; Fresenius Kabi AB; Uppsala, Sweden) or placebo (Ringer’s acetate) as in Study III. Both arterial and venous blood samples, and tissue samples from the brain via craniotomy and the heart via sternotomy were taken as shown in Figure 9. After all samples had been obtained, the animal was euthanised with an i.v. bolus of potassium chloride.
Study V (bupivacaine in pigs)

The study protocol is presented in Figure 10. After obtaining baseline values and samples, the pigs were infused bupivacaine HCl (Bicain®; Orion Pharma, Espoo, Finland) until their MAP decreased to 60% of its baseline value. After all measurements and samples, inhaled isoflurane was discontinued and inspired oxygen was raised to 100%. The pigs were then treated with a 4-ml kg\(^{-1}\) bolus of either 20% lipid emulsion \(n = 7\) or Ringer’s acetate solution \(n = 6\) in 1 min into the central vein in randomised order. Inhaled isoflurane was continued at concentration of 1% when MAP recovered to 60% of baseline level and was raised to 2% when MAP recovered to the baseline level.

Serial needle biopsies (18G Speedcut® biopsy needle; Gallini S.r.l., Mantova, Italy) were obtained from the heart to assay mitochondrial respiration as shown in Figure 10. Similarly, serial arterial blood samples, and tissue samples from the lungs and the apex of the heart were collected for the bupivacaine quantification. An experienced cardiac anaesthesiologist, blinded to the treatment, recorded epicardial

---

**Figure 9. Study IV protocol.**

<table>
<thead>
<tr>
<th>Anaesthesia</th>
<th>Stabilisation</th>
<th>Randomisation</th>
<th>Lipid</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 pigs</td>
<td>15 min</td>
<td>30 min</td>
<td>1 min</td>
<td>29 min</td>
</tr>
</tbody>
</table>

**Figure 10. Study V protocol. FiO\(_2\), fraction of inspired oxygen.**

<table>
<thead>
<tr>
<th>Anaesthesia</th>
<th>Stabilisation</th>
<th>Randomisation</th>
<th>Bupivacaine</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 pigs</td>
<td>2 mg kg(^{-1}) min(^{-1})</td>
<td>1 min</td>
<td>4 ml kg(^{-1})</td>
<td>9 min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prep</th>
<th>13 pigs</th>
<th>4 ml kg(^{-1})</th>
<th>2 mg kg(^{-1}) min(^{-1})</th>
<th>1 min</th>
</tr>
</thead>
</table>

---
Methods

Echocardiography from short axis left ventricular view during the experiment. The left ventricular ejection fraction (EF) was measured from M-mode acquisition using the Teicholz method (Teichholz et al., 1976). At the end of study, the pigs were euthanised with an i.v. bolus of potassium chloride.

**BLOOD AND TISSUE SAMPLE HANDLING**

**Blood samples**
The blood samples (10 ml each) were drawn into heparinised tubes. The plasma was then separated from the whole blood by centrifugation for 10 min at 2500 g, and stored at −22°C. An aliquot of the thawed and carefully mixed plasma samples was used for quantification of the total plasma drug concentrations.

Additionally, an aliquot of the plasma samples was further centrifuged twice for 10 min at 14,000 rounds min⁻¹ (20,800 g) in order to separate the lipid fraction from the aqueous fraction. The un-entrapped drug concentration (non-lipid bound) was then determined in this aqueous fraction.

**Tissue samples**
The tissue samples were rapidly rinsed in saline to remove excess blood, and then dried on filter paper for 5 s. After drying, the samples were wrapped in aluminium foil and stored at −22°C. Before drug concentration measurements in Study IV, approximately 0.5 g of all tissue samples were homogenised with an UltraTurrax T5FU homogenizer (Janke & Kunkel, Staufen, Germany): the brain samples in 4.0 ml and heart samples in 3.0 ml of water. In Study V, the tissue samples (0.3 g) were homogenised with an IKA® UltraTurrax T25 homogeniser (Janke & Kunkel, Staufen, Germany) in 4.0 ml of water. The drug concentrations were further determined using the methods described below.

**Drug concentration quantifications**
The lidocaine concentrations were determined using an Agilent 1100 HPLC (Agilent Technologies, Waldbronn, Germany) coupled to an API 2000 high-performance liquid chromatography–tandem mass spectrometry system (Sciex Division of MDS Inc., Toronto, ON, Canada) using the method described previously by Bo and colleagues (1999). The inter-day coefficients of variation were 1.5% and 3.5% at relevant lidocaine concentrations.

The levobupivacaine and bupivacaine concentrations were determined using an Agilent 1100 HPLC (Agilent Technologies, Waldbronn, Germany) coupled to an API 2000 high-performance liquid chromatography–tandem mass spectrometry system (Sciex Division of MDS Inc., Toronto, ON, Canada) using the method described by Hoizey and colleagues (2005). The inter-day coefficients of variation were 6.9%, 4.5% and 5.4% at relevant levobupivacaine concentrations. For bupivacaine, the in-day coefficients of variation were 10% for plasma samples, 5.4% for heart samples, and 12% for lung samples.

The amitriptyline concentrations were determined using an Agilent 1100 HPLC (Agilent Technologies, Waldbronn, Germany) coupled to an API 2000 high-
performance liquid chromatography–tandem mass spectrometry system (Sciex Division of MDS Inc., Toronto, ON, Canada) the method described by Samanidou and colleagues (2007). The coefficients of variation for plasma samples were 3.7%, 3.1% and 3.5%, and for tissue samples 5.4%, 4.6% and 2.0% at relevant amitriptyline concentrations.

**Mitochondrial respiration assay**
The mitochondrial respiration was assayed using the Oxygraph-2k (OROBOROS Instruments Corp., Innsbruck, Austria). The heart biopsies (mean weight 3.7 mg) were immediately transferred to ice-cold relaxing solution BIOPS (50 mM K+–MES, 20 mM taurine, 0.5 mM dithiothreitol, 6.56 mM MgCl₂, 5.77 mM ATP, 15 mM phosphocreatine, 20 mM imidazole, pH 7.1, adjusted with 5 N KOH at 0 °C, 10 mM Ca–EGTA buffer [2.77 mM CaK₂–EGTA + 7.23 mM K₂–EGTA; 0.1 mM free calcium]) for the transportation (Pesta and Gnaiger, 2011). Before the assays, the heart biopsies were transferred into shredder tubes containing 500 µl of MiR06 respiration buffer (0.5 mM ethylene glycol tetraacetic acid, 3mM MgCl₂·6 H₂O, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH₂PO₄, 20 mM 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid, 110 mM sucrose, 1 g 1⁻¹ BSA, and 280 u ml⁻¹ catalase) (Fasching et al., 2014), and shredded using a SHREDDER SG3 (Pressure BioSciences Inc., South Easton, MA, USA). The shedder tube was then flushed with 2000 µl of MiR06, and a total of 2500 µl of the homogenate was transferred into one of the two Oxygraph-2k chambers for analysis. The biopsies taken at 0 and 10 min time points were analysed simultaneously and the baseline sample in a second round approximately 1 h later.

The oxygen flux per mass was measured during a fatty acid substrate-uncoupler-inhibitor titration protocol (Lemieux et al., 2011; Pesta and Gnaiger, 2011). The substrates were added in the following order: malate (0.8 M, 5 µl) and palmitoyl-L-carnitine (10 mM, 4 µl), adenosine diphosphate (ADP; 0.5 M, 5 µl) and MgCl₂ (0.6 M, 5 µl), glutamate (2 M, 10 µl), succinate (1 M, 20 µl), uncoupler carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP; 1 mM) in three steps of 1 µl, and rotenone (0.2 mM, 2 µl). Oxygen flux was determined after every substrate.

**Statistical analyses**
The statistical analyses were performed using Prism 6.0b–g for Mac OS X (GraphPad Software, Inc., La Jolla, CA, USA).

The incidence rates of LAST were calculated using the fraction of total analysis and the Clopper-Pearson method to calculate 95% confidence intervals, and compared using Fisher’s exact test (I). Variables are presented as mean and standard deviation (SD), median and interquartile range (IQR), and fractions of total with 95% confidence intervals. The differences between treatments were analysed using paired t-test (II–V), unpaired t-test (III–V), Mann-Whitney U test (III–IV), or Wilcoxon matched pairs test (II).
The Holm-Bonferroni method (Holm, 1979) was used to adjust $P$-value for multiple comparisons (II–III). The effect of treatment over time in Study V was tested using two-way analysis of variance with repeated measures with values of the haemodynamic variable over time as within factors, the treatment as the between factor, and their interactions over time. Also Sidak post testing was used. The mitochondrial oxygen flux was analysed using unpaired $t$-test (V).

The drug pharmacokinetics was analysed by calculating the area under the concentration-time curve from 0 to 10, 30 or 60 min ($\text{AUC}_{0-10\text{ min}}$, $\text{AUC}_{0-30\text{ min}}$, or $\text{AUC}_{0-60\text{ min}}$, respectively) and the estimation of its context-sensitive $t_{1/2}$. The AUC of drug was calculated using the linear trapezoidal rule while the $t_{1/2}$ of the drug was determined using non-linear regression.
RESULTS

QUESTIONNAIRE STUDY
A reply was received from all 45 departments, i.e. the response rate was 100%. The total number of regional anaesthetic procedures performed by anaesthesiologists in those departments was approximately 436,300 during the years 2011–2013. After excluding spinal anaesthesias, the total number of regional anaesthesias was approximately 211,700 in the question departments in Finland.

In total, 15 LAST cases were reported for the study period: 4 in university hospitals, 11 in central hospitals and 0 in regional hospitals. When proportioned to the large-dose procedures (spinal blocks excluded), the total incidence (95% confidence interval) of LAST was 0.7 (0.4–1.2) per 10,000 regional anaesthesias, and 0.3 (0.09–0.9) per 10,000 regional anaesthesias in the university hospitals, 1.4 (0.7–2.5) per 10,000 regional anaesthesias in the central hospitals, and 0 (0–2.1) per 10,000 regional anaesthesias in the regional hospitals. The risk for LAST was higher in the central hospitals than in the university hospitals (relative risk [RR] 4.0 [95% confidence interval, 1.3–12; \( P = 0.02 \)). A similar increased risk was seen when all non-university hospitals (regional hospitals included) were compared to the university hospitals (RR 3.3 [95% confidence interval, 1.0–10.3; \( P = 0.04 \)). No deaths due to LAST were reported.

Only one case of cardiotoxicity following regional anaesthesia was reported. This paediatric patient was the only one of the LAST cases during the years 2011–2013 reported to the Register for Adverse Drug Reactions of Finnish Medicines Agency. The patient had developed ventricular tachycardia following a sacral block (bupivacaine with adrenaline) and lipid emulsion had been administered but only after the normalisation of rhythm. The other 14 reported cases were limited to CNS symptoms (seizures). Nine of them underwent interscalene brachial plexus block, two axillary brachial plexus block, two intercostal nerve block and one infraclavicular brachial plexus block. Four of the patients had been treated with lipid emulsion, but only after passing of the acute symptoms. In university hospitals, the anaesthesiologist had used ultrasound guidance in 1 of the 4 cases, and in central hospitals, in 4 of the 11 cases.

In 22 (49%) departments, there was a protocol for the treatment of LAST and lipid emulsion infusion was included in 21 (47% of all departments) of these protocols. In seven (33% of departments with lipid rescue protocol) hospitals, the lipid emulsion was stored in the resuscitation trolley for immediate use.

The internationally recommended commercial lipid emulsion Intralipid® 20% is used in 17 hospitals, three hospitals used ClinOleic® 20%, and one hospital used SMOFlipid® 20%. Six hospitals use the protocol from the Lipidrescue.org website, six the protocol published by ASRA (Neal et al., 2012), one hospital used the protocol published by AAGBI (Cave et al., 2010), and six used a protocol of their own devising.
PLASMA DRUG CONCENTRATIONS

Study II (human study)
Lidocaine plasma concentrations are shown in Figure 11. The mean (SD) peak plasma lidocaine concentrations (C_{max}) were 0.88 (0.21) mg l^{-1} during lipid emulsion and 1.04 (0.29) mg l^{-1} during Ringer’s acetate administration (P = 0.10), and the median (range) time to C_{max} was 5 (1–5) min during both treatments. From 5 min on, the lidocaine concentration decreases with a mean (SD) context-sensitive plasma t_{1/2} of 32 (28) min during lipid emulsion and 30 (19) min during Ringer’s acetate (P = 0.61).

The mean (SD) AUC_{0–30 min} of plasma total lidocaine was 17.4 (4.0) mg min l^{-1} during lipid emulsion infusion and 21.3 (5.2) mg min l^{-1} during Ringer’s acetate infusion (P = 0.10). The mean (SD) AUC_{0–30 min} of un-entrapped lidocaine (16.4 [3.4] mg min l^{-1}) was slightly smaller (5.9%) than that of total lidocaine during lipid emulsion infusion (P = 0.019) and clearly smaller (23%) than that of lidocaine during Ringer’s acetate infusion (P = 0.044).

Study III (levobupivacaine in pigs)
Immediately after its injection, the mean (SD) C_{max} of levobupivacaine was 35.3 (4.6) mg l^{-1} in the Lipid group and 36.3 (11.7) mg l^{-1} in the Ringer group (P = 0.81), from which it decreased rapidly during next minutes (Figure 12). From time point 5 min on, the total plasma concentration of levobupivacaine decreased with a mean (SD) context-sensitive plasma t_{1/2} of 52.2 (10.8) min in the Lipid group, and 57.4 (9.4) min in the Ringer group (P = 0.30).

The mean (SD) AUC_{0–60 min} of plasma total levobupivacaine concentration was 384.4 (92.6) mg min l^{-1} in the Lipid group, and 346.5 (59.7) mg min l^{-1} in the Ringer group (P = 0.32). The mean (SD) AUC_{0–60 min} of un-entrapped levobupivacaine plasma concentration was 383.0 (95.0) mg min l^{-1}, and did not differ from that of plasma total levobupivacaine in the Lipid group (P = 0.67).

Study IV (amitriptyline in pigs)
In Study IV, both arterial and venous amitriptyline concentration decreased by 75–80% indicating distribution of the drug
Results

Figure 1. Mean plasma levobupivacaine concentrations in 10 anaesthetised pigs per group after 3 mg kg$^{-1}$ of levobupivacaine (L) i.v. There were no differences in the areas under the concentration-time curve from 0 to 60 min between the treatments. Error bars show standard deviation.

Figure 12. Mean plasma levobupivacaine concentrations in 10 anaesthetised pigs per group after 3 mg kg$^{-1}$ of levobupivacaine (L) i.v. There were no differences in the areas under the concentration-time curve from 0 to 60 min between the treatments. Error bars show standard deviation.

Figure 13. Mean arterial and venous plasma amitriptyline concentrations in 10 anaesthetised pigs per group after 10 mg kg$^{-1}$ of amitriptyline i.v. Error bars show standard deviation. *P <0.05 between total concentrations in the Lipid and Ringer groups. #P <0.05 between total and un-entrapped concentrations in the Lipid group.
**Study V (bupivacaine in pigs)**

Immediately after bupivacaine infusion, its mean (SD) \(C_{\text{max}}\) was 21.5 (2.5) mg l\(^{-1}\) in the Lipid group and 19.5 (2.9) mg l\(^{-1}\) in the Ringer group (\(P = 0.21\); Figure 14). In the Lipid group, the mean (SD) AUC\(_{0-10}\) of plasma total bupivacaine concentration was 105.2 (13.6) mg min l\(^{-1}\), and 88.1 (7.1) mg min l\(^{-1}\) in the Ringer group (\(P = 0.019\)). The mean (SD) AUC\(_{0-10}\) of un-entrapped bupivacaine, 97.0 (14.5) mg min l\(^{-1}\), was smaller than that of total concentration in the Lipid group (\(P < 0.0001\)), but did not differ from that of the total concentration in Ringer group (\(P = 0.20\)).

**Tissue Drug Concentrations**

**Study IV (amitriptyline in pigs)**

In Study I, the mean (SD) brain amitriptyline concentration was lower 15 min after the end of lipid emulsion infusion (11.5 [2.8] mg kg\(^{-1}\)) than after Ringer infusion (15.3 [4.5] mg kg\(^{-1}\)) (Figure 15; \(P = 0.038\)). In the heart, the corresponding concentrations were 5.1 (1.4) mg kg\(^{-1}\) in the Lipid group and 6.1 (1.2) mg kg\(^{-1}\) in the Ringer group (\(P = 0.086\)).

**Study V (bupivacaine in pigs)**

In Study V, the mean (SD) bupivacaine concentration in the heart was 10.2 (6.2) mg kg\(^{-1}\) in the Lipid group and 12.0 (4.6) mg kg\(^{-1}\) in the Ringer group (\(P = 0.56\)).

---

**Figure 14.** Mean plasma bupivacaine concentrations in six (Ringer group) and seven (Lipid group) anaesthetised pigs after bupivacaine infusion. The area under the concentration-time curve from 0 to 10 min of total bupivacaine was smaller in the Ringer group (\(P = 0.019\)). The area under the concentration-time curve from 0 to 10 min of un-entrapped bupivacaine was smaller than that of total bupivacaine in the Lipid group (\(P < 0.0001\)). Error bars show standard deviation. L, lipid emulsion bolus; R, Ringer’s acetate bolus.

**Figure 15.** Mean brain and heart amitriptyline concentrations in six (Ringer group) and seven (Lipid group) anaesthetised pigs 10 min after bupivacaine infusion. Error bars show standard deviation. *\(P < 0.05\).*
corresponding lung concentrations were 22.5 (3.0) mg kg\(^{-1}\) and 19.7 (7.2) mg kg\(^{-1}\), respectively \((P = 0.38)\).

**HAEMODYNAMICS AND ECG**

**Study II (human study)**

MAP, heart rate and respiratory rate all increased slightly after lidocaine administration (data not shown). Mean heart rate, which increased the most, returned to near baseline value within 5 min. Peripheral oxygen saturation remained unchanged during both treatments. There were no differences between treatments.

**Study III (levobupivacaine in pigs)**

One pig in both groups died in the beginning of rescue treatment in spite of cardiopulmonary resuscitation and adrenaline administration. The mean MAP remained stable after the levobupivacaine and lactic acid infusion even though four pigs (two in each group) developed severe hypotension (in addition to those two pigs that died). Three of those four pigs (two in the Lipid group and one in the Ringer group) were given adrenaline i.v. as MAP decreased below the predetermined value of 25 mmHg. To eliminate the effect of adrenaline, those pigs were excluded from haemodynamic analyses at next two time points. The arterial blood pressure increased similarly in both groups after the beginning of treatment. The mean (SD) peak MAP was 93 (16) mmHg in the Lipid group and 82 (26) mmHg in the Ringer group \((P = 0.34)\). There were also no differences at any later time point.

After the levobupivacaine infusion, the mean heart rate decreased slightly in both groups but began to increase during the lactic acid infusion. The time course of heart rate followed that of MAP returning to near baseline level within 10 min in both groups without differences between the groups.

By the end of hypoventilation period, peripheral oxygen saturation had decreased below 75\% in both groups, after which it normalised rapidly. There were no differences in peripheral oxygen saturation between the groups.

The cardiac conduction times, especially QRS duration, prolonged equally in both groups during the intoxication phase. The median (IQR) time to normalisation of QRS width to baseline was 245 s (198–477 s) in the Lipid group, and 245 s (165–382 s) in the Ringer group \((P = 0.59)\).

**Study IV (amitriptyline in pigs)**

In Study I, the MAP decreased by 20–30\% during the amitriptyline infusion and returned almost to the level before amitriptyline within 30 min. Because the MAP and heart rate were slightly, but significantly higher in the Lipid than Ringer group already at the start of treatment infusions, later values were compared with the 30-min time point values. With the exception of a transient small increase in the Lipid group 5 min after the start of treatment infusions, later values were compared with the 30-min time point values. With the exception of a transient small increase in the Lipid group 5 min after the start of treatment, there were no differences between the groups in the change of MAP (Figure 16). Two pigs became bradycardic and developed severe hypotension during lipid emulsion infusion, and were given adrenaline to prevent cardiac arrest. Their
Results

Figure 16. Median change of mean arterial pressure after the start of rescue infusions. Error bars show 25th to 75th percentile. *P <0.05 between groups.

MAP was considered 25 mmHg from the administration of adrenaline on. Excluding these two pigs, heart rate remained unchanged in both groups after the start of treatment.

ECG showed an equal prolongation of intracardiac conduction times (PQ and QRS duration; data not shown) after amitriptyline infusion. There were no differences in PQ, QRS or QTc times between the groups at any time points.

Study V (bupivacaine in pigs)

Two pigs in the Lipid group and one pig in the Ringer group developed cardiac arrest after bupivacaine infusion and were given cardiac massage until the end of experiment. These pigs were excluded from the haemodynamic analyses. During the echocardiography measurements and cardiac needle biopsy, immediately after bupivacaine infusion, MAP continued to decrease to a mean of 44% of its baseline level until the treatment boluses were given. The treatment-time interaction of MAP was significant (P <0.0001; Figure 17A). Sidak post hoc testing revealed a significantly higher MAP in the Lipid group at time points 2 to 5 min when compared to the Ringer group (P <0.05).

Similar to arterial blood pressure, the treatment-time interaction of systemic vascular resistance index (SVRI) was significant (P <0.0001; Figure 17B). Sidak post hoc testing revealed significantly higher SVRI in the Lipid group at time points 3 to 5 min when compared to the Ringer group (P <0.05).

After bupivacaine infusion, cardiac index decreased similarly in both groups and remained reduced throughout the experiment (Figure 18A). The treatment-time interaction was significant (P = 0.0035), but in Sidak post hoc testing there were no differences between the groups at any time points. Also left ventricular EF decreased after bupivacaine but began to increase similarly after treatment boluses in both groups, and the treatment-time interaction was not statistically significant (P = 0.49; Figure 18B).

ARTERIAL BLOOD GASES

During the hypoventilation phase and i.v. infusion of lactic acid in Study III, all pigs developed hypoxaemia (decreased peripheral oxygen saturation) and hypercapnia. At the end of lactic acid infusion, the blood gas analyses showed severe respiratory and metabolic acidosis (mean pH 7.07 in the Lipid group and pH 7.08 in the Ringer group). There were no differences in arterial blood gas analyses at
Results

Figure 17. A. Time course of mean arterial pressure differed significantly between groups, $P < 0.0001$. B. The time course of mean systemic vascular resistance index differed significantly between groups, $P < 0.0001$. Error bars show standard deviation. In Sidak post-testing ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ between the groups. L, lipid emulsion bolus; R, Ringer’s solution bolus.

Figure 18. A. The time course of mean cardiac index differed between groups $P = 0.0035$. In Sidak post-testing there was, however, no difference between the groups at any time point. B. Mean left ventricular ejection fraction. Error bars show standard deviation. L, lipid emulsion bolus; R, Ringer’s solution bolus.

Baseline or 0 min time point. Within minutes after the end of lactic acid infusion, the acid-base balance began to normalise (Table 9). The normalisation of pH was faster in the Ringer group than in the Lipid group. After statistical correction for
Results

Table 9 Arterial blood gas analyses in Study III

<table>
<thead>
<tr>
<th></th>
<th>Lipid</th>
<th>Ringer</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3 min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.27 (0.07)</td>
<td>7.36 (0.07)</td>
<td>0.005*</td>
</tr>
<tr>
<td>$P_aO_2$ (kPa)</td>
<td>6.4 (1.0)</td>
<td>10.3 (8.8)</td>
<td>0.17</td>
</tr>
<tr>
<td>$P_aCO_2$ (kPa)</td>
<td>7.3 (0.6)</td>
<td>6.1 (1.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Lactate (mmol l$^{-1}$)</td>
<td>5.1 (1.4)</td>
<td>3.7 (1.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Base excess (mmol l$^{-1}$)</td>
<td>-2.9 (4.9)</td>
<td>-0.3 (2.2)</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>5 min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.35 (0.06)</td>
<td>7.42 (0.04)</td>
<td>0.005*</td>
</tr>
<tr>
<td>$P_aO_2$ (kPa)</td>
<td>49.5 (10.4)</td>
<td>47.0 (17.0)</td>
<td>0.70</td>
</tr>
<tr>
<td>$P_aCO_2$ (kPa)</td>
<td>6.1 (0.8)</td>
<td>5.3 (0.7)</td>
<td>0.03</td>
</tr>
<tr>
<td>Lactate (mmol l$^{-1}$)</td>
<td>4.5 (1.1)</td>
<td>3.7 (1.4)</td>
<td>0.19</td>
</tr>
<tr>
<td>Base excess (mmol l$^{-1}$)</td>
<td>-0.7 (2.7)</td>
<td>1.0 (2.6)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Values are means (standard deviations). *P value significant after Holm–Sidak correction. $P_aCO_2$, arterial carbon dioxide pressure; $P_aO_2$, arterial oxygen pressure. Modified from Study III.

multiple comparisons, BE, lactate or $P_aCO_2$ did not, however, differ at any time point.

MITOCHONDRIAL RESPIROMETRY

In Study V, the analysis of mitochondrial respiration revealed a significantly higher oxygen flux after the following substrates in the Lipid group than in Ringer group: glutamate, succinate, FCCP, and rotenone (Figure 19). There were no differences between the mitochondrial oxygen flux at end of bupivacaine infusion and that at baseline at any step of the respirometry protocol.

CENTRAL NERVOUS SYSTEM TOXICITY

Electroencephalography

In Study II, EEG spectral power at baseline was comparable between study phases. The power in delta, alpha and beta bands increased similarly after lidocaine during both treatments. The median percentual changes in the delta power band, which was most affected, ranged from 37% to 72% during lipid emulsion, and from 15% to 54% during Ringer’s acetate without

![Figure 19. Mean cardiac mitochondrial respiration of the pigs was stimulated after lipid emulsion administration when compared to Ringer’s solution (*P < 0.05) through complex I after glutamate (Glu), through complex I and II after succinate (Suc), and after uncoupling, through complex I and II (FCCP) and complex II alone after rotenone (Rot). Error bars show standard deviation. M, malate; Pal, palmitoyl-L-carnitine; ADP, adenosine diphosphate; FCCP, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone.](image-url)
Results

...differences between the study phases. Theta power band remained unaffected. Based on visual evaluation, there were no epileptiformic disturbances present in any of the EEG recordings.

Subjective symptoms
During both phases of Study II, all volunteers reported clear symptoms of lidocaine CNS toxicity. The toxicity symptoms (numbness mouth/tongue, tingling mouth/tongue, metallic taste, auditory disturbance, dizziness/lightheadedness, incoherence, sensation of twitching) began within a minute after lidocaine administration. After summing the symptoms for each volunteer, there were no difference between treatments: \( P = 0.98 \) at 1 min, \( P = 0.88 \) at 2 min, \( P = 0.75 \) at 3 min, \( P = 0.81 \) at 4 min, and \( P = 0.50 \) at 5 min. Almost all sensations of CNS toxicity resolved within 5 min. Seven volunteers during lipid emulsion and six volunteers during Ringer’s solution had visible fasciculations in the eyelids or extremities ending within two minutes after lidocaine administration. No other objective signs of CNS toxicity were noted.

Adverse effects
In Study II, four of the eight volunteers reported pain in the arm into which lipid emulsion had been administered, and it lasted for approximately 30 min. There was no pain during the Ringer phase of the study.

Four pigs in Study III developed mild redness of the skin during lipid emulsion infusion. Similar redness was seen also in three pigs in Study IV, and in three pigs in Study V. The redness was not accompanied with particular haemodynamic instability or with changes in airway pressures.
**DISCUSSION**

**MAIN FINDINGS**
The incidence of LAST in Finland was very low (I). With only 15 cases of LAST during the years 2011–2013, the total incidence was 0.7 per 10,000 regional anaesthesias (excluding spinal blocks). After dividing the hospitals by their level, the incidence proved to be four times higher in the central hospitals than in university hospitals. LAST was most rare in regional hospitals but the total number of regional anaesthesias was also much lower in those hospitals. Even though the efficacy and mechanisms of action of lipid rescue remain uncertain, lipid emulsion has been adopted as a part of treatment of LAST in 47% of Finnish anaesthesia departments.

The ability of lipid emulsion to entrap drugs in the plasma is drug and dose dependent. The most lipophilic drug studied, amitriptyline (approximately 20 times more lipophilic than bupivacaine or levobupivacaine), was both entrapped to arterial plasma and drawn away from critical tissues, especially from brain (IV). Local anaesthetics, such as lidocaine and levobupivacaine (II–III), seem not to be lipophilic enough to be entrapped by lipid emulsion used in clinically recommended doses (Cave *et al.*, 2010; Neal *et al.*, 2012). However, when the lipid emulsion dose was increased, bupivacaine was entrapped into plasma (V). Lipid emulsion affected the lidocaine pharmacokinetics by increasing $V_d$ in healthy volunteers (II).

The CNS toxicity symptoms of lidocaine could not be prevented or alleviated by using lipid emulsion (II). EEG revealed an increased power especially in the delta band as a mark of disturbance of cortical function (Smith, 2005) after lidocaine administration without differences between lipid emulsion and placebo treatment. Also the severity or duration of subjective symptoms did not differ between rescue treatments.

Complex I and II mediated cardiac mitochondrial respiration was stimulated by administration of lipid emulsion (V). This stimulated function was not, however, accompanied by improved cardiac function as measured as cardiac index or left ventricular ejection fraction. Improved haemodynamic recovery could be demonstrated only in Study V using a large lipid emulsion dose. This improved recovery was associated with increased peripheral vascular resistance and a rise of arterial blood pressure after lipid emulsion. In Study III, there were no differences in haemodynamic recovery, and in Study IV, the haemodynamics were near normal by the start of treatment infusions.

**THE EXPERIMENTAL MODEL COMPARED TO A CLINICAL SITUATION**
A typical case of LAST occurs after unintentional intravascular injection of the drug (Ecoffey *et al.*, 2014). The more uncommon cause of LAST is absorption of the drug from tissues after a substantial dose (Whiteman and Kushins, 2014). The clinical presentation of LAST in published cases has ranged from mild CNS toxicity (Espinet and Emmerton, 2009; Mizutani *et al.*, 2011) to seizures (Calenda and Dinescu, 2009; Whiteside, 2008) and to cardiotoxicity unresponsive to treatment (Litz *et al.*, 2006; Rosenblatt *et al.*, 2006). Seizures, that
Discussion

55

typically precede cardiac symptoms, can rapidly render the patient hypoxic and acidotic (Dernedde et al., 2004; Rosenberg et al., 1983; Tuominen et al., 1991). Acidosis ionises the basic local anaesthetic molecules and increases both the amount of protein-unbound drug concentration (Coyle and Park, 1985) and the intracellular binding of local anaesthetics to sodium channels (Scott, 1986), which worsens the systemic toxicity (Heavner et al., 1992; Porter et al., 2000; Rosen et al., 1985). To mimic less severe symptoms of LAST after rapid i.v. injection of local anaesthetic, a bolus dosing was used in Studies II and III. In Study III, the infusion of lactic acid after local anaesthetic injection simulated those aforementioned typical pathophysiological changes caused by seizures. In Study V, the infusion of bupivacaine until MAP decreased to 60% of its baseline level resembled more a case of substantial rapid absorption of bupivacaine from tissues. The MAP decrease to 60% was chosen because in pilot studies, MAP continued to decrease during the echocardiography measurements and mitochondrial biopsy sampling until treatment began and lower target MAP would probably have lead to cardiac arrest. Even with current doses in Studies III and V, some pigs developed cardiac arrest and could not be resuscitated.

In clinical cases of amitriptyline poisoning the drug is taken orally after which it absorbs gradually from the gastrointestinal tract. Often part of the ingested drug is still unabsorbed at the time of hospital admission and activated charcoal can be used to prevent the further absorption. In the experimental model of Study IV, amitriptyline was, however, given i.v. as an infusion to standardise the drug dose and to avoid the inter-individual differences in gastrointestinal absorption. To mimic a clinical situation in which part of the drug has already been absorbed, its further absorption is prevented, and the absorbed drug has mainly distributed into vital organs, such as brain and heart, amitriptyline was let to distribute for 30 min after the infusion. The plasma concentrations after the infusion of 10 mg kg\(^{-1}\) amitriptyline in our pigs were comparable to clinical situations of amitriptyline poisonings in humans (Hultén et al., 1991). The brain and heart amitriptyline concentrations in our pigs were also comparable to the post-mortem tissue concentrations after fatal amitriptyline poisonings in humans (Bynum et al., 2005; Musshoff et al., 2004; Tracqui et al., 1990).

Since it is unethical to give toxic drug doses to humans, animal models were adopted to mimic clinical intoxications in humans. To minimise the inter-species differences, all animal studies were conducted using pigs which closely resemble humans (see ‘Limitations’ below, page 62).

LIPID EMULSIONS

The fatty acids in the lipid emulsion can be divided into short (up to 4 atoms in the carbon chain), medium (6 to 12 carbon atoms) and long (over 12 carbon atoms). The lipid emulsion used in Studies II–V was Intralipid® which consists of long-chain triglycerides derived from soya bean oil. The triglyceride composition can, however, vary greatly between different lipid
emulsions as they can be derived from, for instance, olive, coconut or fish oils (Carpentier and Dupont, 2000). Another clinically widely used lipid emulsion, ClinOleic®, is mainly composed of olive oil based triglycerides and to a lesser degree of soya bean oil based (Calder et al., 2010) while the triglycerides of another clinical lipid product SMOFLipid® are coconut, soya bean, olive and fish oil derived (Calder et al., 2010). Therefore, the main triglyceride in Intralipid® is linoleic acid, in ClinOleic® oleic acid, and SMOFLipid® consists more evenly of several triglycerides than the other two. In addition to different triglyceride compositions between different lipid emulsions, the exact triglyceride concentrations vary also in the oils they are derived from (Fatemi and Hammond, 1977), which causes a variation in the final product (Fresenius Kabi AB, 2013). Even though the composition affects the stability of the lipid emulsion and even the immunologic responses (Calder et al., 2010), comparative studies have shown no marked difference in rescue potential of different lipid emulsions in LAST (Bonfim et al., 2012; Candela et al., 2010; Udelsmann and Melo, 2015), and the present treatment guidelines do not distinguish any lipid emulsion from others (Cave et al., 2010; Neal et al., 2012).

The recommended dosage of lipid emulsion in lipid rescue for LAST is 1.5 ml kg\(^{-1}\) bolus in 1 min followed by 0.25 ml kg\(^{-1}\) min\(^{-1}\) infusion (Cave et al., 2010; Neal et al., 2012). For a 70-kg patient, the bolus dose would be approximately 100 ml which is approximately the highest volume that can be given through a peripheral 18G cannula in 1 min. However, in most experimental studies the lipid emulsion dose has been much higher, varying from 4 to 10 ml kg\(^{-1}\) (Fettiplace et al., 2015; Fettiplace, Akpa, et al., 2014; Hicks et al., 2009; Udelsmann and Melo, 2015). Such high volumes would likely be impossible to administer to clinical patients though peripheral i.v. routes in a rapid manner.

**Liposomes**

In addition to lipid emulsions, various lipid nanoparticles, such as liposomes (100–200 nm) and lipid nanocapsules (~100 nm), have been developed for treatment of severe intoxication of lipophilic drugs (Dhanikula, Khalid, et al., 2007). Liposomes consist usually of phospholipid bilayers that form a spherical lamellar particle (Damitz and Chauhan, 2015). To enhance the effect of liposomes, the pH of the inner aqueous part can be modified to cause ionisation of the drug within the liposome. After ionisation, the drug cannot anymore permeate the lipid bilayer. The liposomes can also be charged negatively to increase their interaction with positively-charged drugs (Damitz and Chauhan, 2015). Therefore, the basic principle is the same as in the case of lipid emulsion: to create liposomes that entrap the lipophilic molecules as much as possible in order to prevent their action.

These liposomes also seem to be more effective in entrapping bupivacaine compared to lipid emulsions (Litonius, Lokajová, et al., 2012). In some cases liposomes could be used also as an antidote carrier (Damitz and Chauhan, 2015). There are, however, possible safety issues before the liposomes can be introduced to clinical practice as some liposomes may also cause
haemodynamic instability (Litonius, Lokajová, et al., 2012). The nanosized liposomes have shown some promising effects in entrapment of amitriptyline (Dhanikula, Lamontagne, et al., 2007; Howell and Chauhan, 2008) and treatment of clomipramine poisonings (Cave et al., 2013).

The treatment of poisonings is not the only medical application for liposomes. The most important use for these liposomes is to act as drug delivery systems. They are used in delivery of various drugs, e.g. cytostatics, anti-fungals, and antibiotics (Allen and Cullis, 2013). In the field of anaesthesiology, they have also shown promise in prolonging the action of epidural anaesthesia if used as a drug carrier (Leng et al., 2012).

**HAEMODYNAMIC EFFECTS OF THE LIPID EMULSION**

The results of the present study (II–V) indicate that the clinically recommended lipid emulsion dose of 1.5 ml kg\(^{-1}\) followed by infusion of 0.25 ml kg\(^{-1}\) min\(^{-1}\) (Cave et al., 2010; Neal et al., 2012) may be too small to produce any resuscitative effect – at least in case of LAST. The lack of effect of the recommended dose has been shown also earlier (Litonius et al., 2012b; Litonius, Tarkkila, et al., 2012) while higher doses from 4 ml kg\(^{-1}\) on have shown promising results on haemodynamic recovery and pharmacokinetic effects (Candela et al., 2010; de Queiroz Siqueira et al., 2014; Fettiplace, Akpa, et al., 2014; Shi et al., 2013; Udelsmann and Melo, 2015). The beneficial effects of the higher lipid emulsion doses are likely not due to entrapment. Even theoretically, lipid emulsions should entrap local anaesthetics only marginally (Damitz and Chauhan, 2015).

In Study V, the administration of 4 ml kg\(^{-1}\) of lipid emulsion, 2.7 times higher bolus dose than clinically recommended in LAST, increased peripheral vascular resistance, which caused a rapid increase in arterial blood pressure. Similar findings in another recent pig study support the role of vasoconstriction as an important mechanism of action of lipid rescue (Udelsmann and Melo, 2015). Also in rats, the administration of lipid emulsion increases the carotid resistance in bupivacaine intoxication (Fettiplace, Akpa, et al., 2014). Injection of massive lipid emulsion dose, 9 ml kg\(^{-1}\), alone caused a similar rapid increase of arterial blood pressure as in Study V, and increased carotid flow (Fettiplace et al., 2013). In the present study, there were no signs of inotropy or improvement of cardiac function, i.e. cardiac index or left ventricular EF.

It is possible that the haemodynamic effects of lipid emulsion are mediated by \(\alpha_1\)-adrenoceptors. In human volunteers, the \(\alpha_1\)-adrenergic receptor reactivity increases after much lower doses of lipid emulsion than used in Study V (Haastrup et al., 1998; Stepniakowski et al., 1996), and even a slow infusion of lipid emulsion gradually increases arterial blood pressure (Kearney et al., 2002; Stojiljkovic et al., 2001). In rat aorta rings, lipid emulsion similarly increases the reactivity to noradrenaline after local anaesthetic induced vasodilatation (Lee et al., 2013), and can
alone reverse the levobupivacaine induced vasodilatation (Ok, Park, et al., 2013).

α₁-adrenoceptors are G-protein-coupled receptors of which activation mediates, for instance, vasoconstriction. In the cell, the activation of the receptor stimulates phospholipase C activity which then through phosphoinositol pathway activate protein kinase C (García-Sáinz et al., 2000). For instance oleic acid, which is one of the main components of Intralipid®, (see Table 4, page 22), can directly activate protein kinase C (Murakami and Routtenberg, 1985). The increase in blood pressure after oleic acid infusion can be prevented with prazosin, an α₁-receptor blocker (Grekin et al., 1997). In addition to protein kinase C-mediated effects, oleic acid and palmitic acid, other components of Intralipid®, can inhibit the activation of endothelial nitric oxide synthase (eNOS) independently of protein kinase C activation (Davda et al., 1995; Kim et al., 2005). Therefore, Intralipid® can directly cause vasoconstriction while it also prevents the formation of vasodilative nitric oxide.

On the other hand, Intralipid® itself contains small amounts of arachidonic acid and the main component of Intralipid®, linoleic acid, is also converted to arachidonic acid (Salem et al., 1999). The metabolism of arachidonic acid by cyclooxygenase-1 (Brock et al., 1999) and cytochrome P450 (Rahman et al., 1997) produces several vasoactive metabolites that can partly explain the increased peripheral resistance.

**MITOCHONDRIAL EFFECTS OF LIPID EMULSION**

In contrast to previous findings from isolated mitochondria (Cela et al., 2010; Sztark et al., 1998), bupivacaine did not significantly affect mitochondrial respiration even though MAP decreased to 60% of its baseline level, and both cardiac index and EF decreased markedly. The depression of complex I function by 50% occurred, however, at much higher bupivacaine concentration of 0.38 mM, i.e. 110 mg l⁻¹, than in Study V. As the complex I-mediated respiration of isolated mitochondria was also not altered after a 10-mg kg⁻¹ bupivacaine dose in rats (Partownavid et al., 2012), it is likely that the mitochondrial effects of bupivacaine are not relevant at bupivacaine concentrations during clinical LAST.

Even though the mitochondrial respiration remained unaltered after giving bupivacaine, administration of lipid emulsion caused a significant increase in oxygen flux after the addition of substrates glutamate and succinate, uncoupler FCCP, and complex I inhibitor rotenone, while it remained unaltered when treatment consisted of Ringer’s acetate administration. In the aforementioned rat study (Partownavid et al., 2012), there was no increment in complex I-mediated respiration after administration of lipid emulsion. They did not, however, include any substrates of fatty acid oxidation as we did in Study V. The mitochondria were also isolated before respirometry, which may have affected the results. According to the present results it seems possible that lipid emulsion slightly increases the cardiac
mitochondrial respiration – even though it was not accompanied with improved cardiac function.

**ENTRAPMENT OF DRUGS BY LIPID EMULSION**

Theoretically, the predicted entrapment of amitriptyline by lipid emulsion is very good (Damitz and Chauhan, 2015). This was also supported by the present studies as the only drug significantly entrapped by lipid emulsion after clinically recommended rescue doses was amitriptyline (IV). A similar increase in blood amitriptyline concentration has also been shown previously in both pigs and rats but without any haemodynamic effects (Litonius *et al*., 2012a; Perichon *et al*., 2013). In Study IV, the entrapment was accompanied by a favourable resuscitative effect, i.e. lipid emulsion reduced amitriptyline concentration in the brain. Tissue TCA concentrations have not been measured in the other studies to confirm the present findings, however.

All local anaesthetics used seem not to be lipophilic enough to be entrapped by lipid emulsion (II, III). Even during acidosis, which increases the free local anaesthetic fraction, lipid emulsion had no effect on levobupivacaine concentrations (III). Only after a higher lipid emulsion dose, bupivacaine was slightly entrapped (V). The entrapment of local anaesthetics by lipid emulsion has been only marginal also in previous studies in which clinically recommended lipid emulsion doses has been used (Litonius *et al*., 2012b; Litonius, Tarkkila, *et al*., 2012). Much higher lipid emulsion doses can, however, increase the blood total local anaesthetic concentration (Fettiplace *et al*., 2015). Such high lipid emulsion doses (10 to 15 ml kg⁻¹) can also affect the tissue local anaesthetic concentrations (Fettiplace *et al*., 2015; Shi *et al*., 2013), which did not seem to occur even after the 4-ml kg⁻¹ dose used in Study V.

Originally, the *lipid sink* effect was considered the main mechanism of action of lipid rescue. At the moment, *lipid sink* seems to be only a minor mechanism of action, and may not be important at all in case of LAST.

**LIPID RESCUE – MULTIPLE SUGGESTED RESUSCITATION MECHANISMS OF ACTION**

Intralipid®, as well as other lipid emulsions, is a combination of several components. The amounts of the components can also vary between packages (see Table 4, page 22). The vasoconstrictive effects, as well as other possible positive effects, of lipid emulsions in treatment of drug intoxication are likely mediated by specific fatty acids, such as linoleic, oleic or palmitic acid. Even though the fatty acids may not be effective enough to cause marked vasoconstriction themselves, they could be used to sensitisise α₁-adrenoceptors to, for instance, noradrenaline (Haastrup *et al*., 1998; Lee *et al*., 2013; Stepniakowski *et al*., 1996) after local anaesthetic has induced vasodilatation. Such sensitisation of α₁-adrenoceptors may also explain the recovery of some patients to whom lipid emulsion had been administered in addition to adrenoceptor agonists (Harvey *et al*., 2011; Wong *et al*., 2010).
Long-chain fatty acids, e.g. oleic, linoleic and palmitic acids, seem to increase the cardiac calcium concentration (Huang et al., 1992), and therefore, improve cardiac function. Such cardiac effects could not, however, be demonstrated in the present study (V). The influence on calcium concentration seems, however, to be important in treatment of LAST as amrinone, a pyridine phosphodiesterase 3 inhibitor, which inhibits the breakdown of cyclic adenosine monophosphate (cAMP) and increases intracellular calcium concentration, improves the recovery from bupivacaine cardiotoxicity (Lindgren et al., 1992). On the other hand, a calcium sensitiser levosimendan does not markedly improve the recovery from bupivacaine intoxication (Aittomäki et al., 2010). Apparently, direct calcium agonists would be more effective than lipid emulsion alone. It is, however, possible that lipid emulsion could enhance the effects of amrinone and even levosimendan.

As mentioned before, lipid emulsion acts as a source of vasoactive prostaglandins which may also play a role in lipid resuscitation; part of the prostaglandins act as vasoconstrictors and part as vasodilators (Skeie et al., 1988). Therefore, it could be possible to administer only these vasoactive prostaglandins instead of lipid emulsion. However, lipid emulsion -induced vasoconstrictive effects are not necessarily positive as lipid emulsion increases also pulmonary vascular resistance at least in neonates (Prasertsom et al., 1996). In pigs and sheep, a similar pulmonary vasoconstriction caused by lipid emulsion has been linked to vasoactive prostaglandins (Bedocs et al., 2014; McKeen et al., 1978). Thus, the vasoconstrictive effects on the pulmonary circulation may limit the applicability of prostaglandins to resuscitation use as they may impair the oxygenation of blood in the lungs – at least in injured lungs (Lekka et al., 2004; Skeie et al., 1988). In humans, there are, however, no studies on lung function after the higher lipid emulsion doses used in lipid rescue.

ADVERSE EFFECTS OF LIPID EMULSION
As already mentioned in the ‘Adverse effects’ paragraph (see page 33), administration of lipid emulsion can potentially cause several different adverse reactions, e.g. hypersensitivity reactions, ARDS or pancreatitis. In the only controlled human study before Study II, such effects were not noted (Litonius, Tarkkila, et al., 2012). The only adverse effect was increased saliva production of one volunteer. In Study II, there were no other adverse effects than pain in the infusion arm during lipid emulsion administration. The total number of volunteers in these two studies, n=16, cannot, however, exclude a possibility of other more serious but less frequent complications.

In all pig studies (III–V), some of the animals developed mild mottling of the skin during or after lipid emulsion administration. Some researchers have suggested that the skin reactions may be related to complement activation (see ‘Limitations’ paragraph below). In none of the present studies, skin reactions were accompanied with haemodynamic instability or increased airway pressures suggesting that the skin reactions were not
related to any anaphylactoid or anaphylactic reactions. In human volunteers (II), redness of the skin was not observed during lipid emulsion administration.

**LAST COMPARED TO OTHER COMPLICATIONS OF ANAESTHESIA**

The incidence of serious LAST in Finland during the three survey years was very low, only 0.7 per 10,000 regional anaesthesias which could potentially cause LAST (I). Also in France and the US, the incidence of LAST has ranged from 0.6 to 1.5 per 10,000 procedures after excluding spinal anaesthesias as in our study (Auroy et al., 2002; Ecoffey et al., 2014; Sites et al., 2012). Barrington and colleagues (2013) reported that the incidence of serious LAST was slightly higher, 3.5 per 10,000 peripheral nerve blockades, in Australia. There is, however, some variation between the study settings as in most of the other studies only particular procedures were included (e.g. axillary brachial plexus block, or all peripheral nerve blockades) making the direct comparison of incidences difficult.

Death is a serious complication in medical practice. The incidence of death related to and primarily due to anaesthesia has been declining during the last 60 years (Bainbridge et al., 2012; Tikkanen, 1992). Within the last two decades, the overall incidence of death due to anaesthesia has ranged from 5 to 21 per one million anaesthesias between different studies (Aitkenhead, 2005; Lienhart et al., 2006). Schiff and colleagues (2014) showed very recently that in otherwise healthy patients (American Society of Anesthesiologists Physical Status I–II), a death directly involving anaesthesia is very uncommon, 0.7 per one million procedures. The only fatality directly involving anaesthesia in that study was due to a failed intubation. The number of anaesthesia-related deaths is, however, higher than that; approximately 10% of all anaesthesia-related deaths are primarily due to anaesthesia or anaesthetics (Li et al., 2009). The causes of fatalities due to anaesthesia or anaesthetics can be, for example, total spinal block by local anaesthetics (Auroy et al., 2002; Pitkänen et al., 2013), respiratory failure due to impossible intubation (Lienhart et al., 2006), or hypovolemia (Lienhart et al., 2006).

LAST is not the only complication related to local anaesthetics and regional anaesthesia. Rarely, local anaesthetics can cause IgE-mediated, but also other types of, allergic reactions, even anaphylaxis (Bhole et al., 2012). Similarly, local reactions, such as skeletal muscle destruction (Zink and Graf, 2004), are rare in clinical setting. More common are technical complications, i.e. bleeding or nerve damage caused by needles or catheters. An analysis of closed claims in Finland revealed that the incidence of neuraxial haematomas, which can cause, for instance, paraplegia, was 0.1 per 100,000 spinal anaesthesias and 0.4 per 10,000 epidural anaesthesias (Pitkänen et al., 2013). One quite typical complication of peripheral nerve blocks is neuropathy, e.g. paraesthesia or even neuropathic pain. Depending on the block type, the incidence of long-lasting neuropathy range from 0 to 30 per 10,000 blocks (Auroy et al., 2002).
LIMITATIONS
The questionnaire (I) was made retrospectively and was based mainly on the memory of the department chiefs. It is possible that some cases of LAST were not reported to them, and therefore, later to us. The anaesthesia departments are, however, relative small in Finland, and it is unlikely that these potentially life-threatening complications would not have been reported to the department chief. Very similar incidence rates of serious LAST from other countries support the results of the present study (Auroy et al., 2002; Barrington and Kluger, 2013; Ecoffey et al., 2014).

In Study II, the lipid emulsion bolus was given before the lidocaine injection, which differs from a clinical situation. The CNS effects of 1 mg kg\(^{-1}\) lidocaine are, however, short-lived, and passed within 5 min in that study. To maximise the effect of lipid emulsion, the bolus was given in advance.

All animal studies (III–V) were conducted under general anaesthesia with isoflurane while all patients are unanaesthetised at the time of amitriptyline ingestion. Also, most of the patients undergoing regional anaesthesia are sedated at the most. General anaesthetics have shown to protect against, for instance, arrhythmias (Warltier et al., 1988), and they reduce the systemic toxicity of local anaesthetics (Copeland et al., 2008). This might explain why serious arrhythmias were not noted. The level of anaesthesia was, however, similar in both groups in all studies, and the isoflurane concentration was kept as low as possible at 2.0±0.1% to minimise its effects.

Some researchers have claimed that the pig is unsuitable for lipid rescue research (Weinberg and Rubinstein, 2012). A reason for the criticism is that pig may develop complement activation related hypersensitivity reactions (pseudoallergy, CARPA) to certain liposomes (Szebeni et al., 2007). Such reaction may, however, develop also in humans and pig can be used as a model of CARPA reactions (Szebeni et al., 2007). Even though such complement activation cannot be demonstrated in pigs after lipid emulsion administration, they seem prone to develop haemodynamic instability after high doses of lipid emulsion (Bedocs et al., 2014). The lipid emulsion doses used in the present study were, however, smaller, and no particular haemodynamic instability was noted. Nevertheless, even though pigs are not physiologically identical to humans, they are considered to be a most suitable animal model for both physiological and pharmacological research (Swindle et al., 2012; Yealy, 1993). The pigs resemble humans also anatomically, and from a practical point of view, all catheters and anaesthesia equipment for human use are also suitable for pigs weighing 20 to 30 kg. On the other hand, widely in lipid rescue research used rats have a species-specific ability to recover spontaneously from ventricular fibrillation (Yealy, 1993). Even after cardiac arrest for several minutes the heart can be restarted by mechanical compression, which does not happen in pigs and humans (Yealy, 1993). This ability of spontaneous recovery might have helped the more favourable results in rats than in pigs.
**CONCLUSIONS**

The following conclusions can be made based on Studies I–V:

1. The total incidence of local anaesthetic systemic toxicity in Finland is low – only 0.7 per 10,000 regional anaesthesias, and the incidence of cardiac toxicity is much lower (I). Lipid rescue is adopted by less than half of the Finnish anaesthesia departments (I).

2. Lipid emulsion does not alleviate CNS toxicity symptoms of lidocaine in human volunteers (II). In pigs, lipid emulsion does not improve recovery from levobupivacaine intoxication which is exacerbated by acidosis and hypoxaemia as in clinical situation (III).

3. Local anaesthetics are not entrapped after intravenously administered lipid emulsion in clinically recommended doses and much higher lipid emulsion doses are probably needed for any significant entrapment (II, III, V). On the other hand, in clinically relevant doses lipid emulsion entraps amitriptyline in plasma to some degree even after distribution of amitriptyline (IV). In addition, lipid emulsion can also reduce tissue amitriptyline concentration to a slight degree (IV).

4. In pigs, a higher lipid emulsion dose than clinically recommended improves recovery from bupivacaine cardiac toxicity through peripheral vasoconstriction, and slightly stimulates mitochondrial complex I and II mediated respiration (V). The improvement of mitochondrial function does not, however, correlate with cardiac function (V).
**FUTURE IMPLICATIONS**

This study has enlightened the uncertainty about the possible mechanisms of action of lipid rescue. According to this thesis, the effects of lipid emulsion do not emerge until a larger dose is used than recommended. The dose of 4 ml kg$^{-1}$, at least in 1 min, is too high to be administered to clinical patients through peripheral cannulas that do not allow such administration rates. Therefore, we need more comprehensive dose dependence studies to confirm the optimal dosage of lipid emulsion. On the other hand, we need also more comprehensive studies on the safety of the larger doses of lipid emulsion.

Randomised clinical trials on lipid rescue of LAST are difficult, or even impossible, to organise as the incidence of LAST is so low and it would be impossible for the patient to give a written consent to the study in an emergency situation. Symptoms of LAST also typically ease rapidly and treatment of, for instance, convulsions with other medication is essential, which impedes the evaluation of the efficacy of lipid emulsion. However, more human volunteer studies could be possible to conduct with local anaesthetic doses that are known to be safety. On the other hand, in cases of ingestion of other drugs, such as amitriptyline or other lipophilic psychopharmaca, it could be possible to study the efficacy of lipid rescue in a clinical setting in the emergency departments.

The best animal model for lipid rescue still remains uncertain. On the one hand, the physiology and anatomy of rodents differ greatly from humans, while on the other hand, pigs may be more sensitive to especially higher lipid emulsion doses than rodents. The effects of high lipid emulsion doses in humans are not known. We experienced pig a practical animal model for lipid rescue studies, but it may still be reasonable to find out the best of them.

The originally proposed mechanism of action of lipid rescue, *lipid sink*, cannot account for the main mechanism of action and explain the potential positive effects seen in clinical case reports. In this thesis, two other mechanisms of action, vasoconstriction and slightly stimulated mitochondrial respiration, were shown. To fully understand the efficacy of lipid emulsion, we still need to dig more deeply into the mechanisms and even to study new possibilities, such as prostaglandins.

In addition to studies on mechanisms of action of lipid rescue, more specific liposomes could prove also more effective than the present lipid emulsions. Liposomes can be modified to maximise, for example, the *lipid sink* effect, but possibly also other effects could be built into them.

As lipid emulsion does not prove to be a miraculous antidote for LAST, we still lack a specific treatment for local anaesthetic intoxication and it remains a potentially fatal complication. Therefore, we need to focus also on the training of both young and also more experienced anaesthesiologists. The most important aspects are the usage of small test doses, aspiration, usage of ultrasound, and of course, the early recognition of the symptoms of LAST. The best treatment for LAST is to prevent them.
As LAST is, luckily, a rare complication of regional anaesthesia, we would need an international, e.g. Scandinavian or European, registry into which all cases could be reported. Such registry would provide more information about the circumstances leading to LAST, which would be useful in the training of anaesthesiologists.

Education, monitoring, rapid intervention are no new means for safe performance of regional anaesthesia but their role needs to be reemphasised.
ACKNOWLEDGEMENTS

These studies were conducted in the Departments of Anaesthesiology, Intensive Care and Pain Medicine, and Clinical Pharmacology of University of Helsinki during the years 2012–2015. This whole project would not have been possible without the help and support of many important people.

I am most grateful to my supervisors Erik Litonius, MD, PhD, and Professor Janne Backman. Thank you for your help, guidance and supervision through this whole project. Erik, you have guided me from the very first steps of my researcher career and taught me a lot, beginning from all the practical things one must know of doing research. Janne, it has been a privilege to be supervised by you. Your expertise in the field of pharmacology has been essential for this whole project.

The official reviewers, Professor Riku Aantaa and Professor emeritus Pauli Ylitalo, helped me to improve this thesis with their critique and suggestions.

I also wish to thank Professor emeritus Per Rosenberg for being my ‘third supervisor’ as the head of the research group. It has been a privilege to work under your guidance. Your endless time for me and your huge experience were crucial to all the original studies but also to this thesis.

I gratefully acknowledge the work of Jari Kainulainen with the EEG recordings in Study II, and the technical help of Päivi Turunen in Studies I and II. I wish also thank Veikko Huusko and Olli Valtanen for their technical assistance in Studies III–V. I am very grateful to Jouko Laitila and Lisbet Partanen for quantifying the drug concentrations in Studies II–V. Nada Bechara-Hirvonen made the mitochondrial assays in Study V, which is also deeply acknowledged.

I would also like to thank my co-authors Professor emeritus Pertti Neuvonen, Docent Tapani Salmi, Juhani Haasio, MD, PhD, Docent Pekka Tarkkila, Docent Markus Skrifvars, Docent Mikko Pitkänen, Alexey Schramko, MD, PhD, and Professor Eero Mervaala for their contributions in the original studies.

I would like to thank the volunteers who participated in Study II.

This work was financially supported by research grants from Liv och Hälsa Foundation, Stiftelsen Dorothea Olivia, Karl Walter och Jarl Walter Perkléns Minne, Suomen Lääketieteen Säätiö, and Finska Läkaresällskapet, and Helsinki University Central Hospital research funds.

I wish to thank all my friends for their encouragement and peer support. Also, it has been essential to get opportunities to lay all this aside and do something else!

I am very grateful to my parents, Päivi and Heimo, for encouraging me to choose the career and life I dreamed of. You have made me believe that nothing is impossible. Without your support and trust,
I would not have gone so far. I wish also to thank my brothers, Esko and Timo, for the great time we have had over the years.

Our son Olavi, you are the most marvellous thing of all. Your charming smile keeps on cheering me up day after day. It will be great to see you grow and learn things!

Finally, I would like to thank my lovely and beautiful wife Veera. You supported me and believed in me all the time even though I doubted whether this project would ever end. You made this possible. I love you.
REFERENCES


Engel PT, Davidow JS. Intravenous fat emulsion to reverse haemodynamic instability from intentional...


References


Lee HMD, Archer JRH, Dargan PI, Wood DM. What are the adverse effects associated with the combined use of intravenous lipid emulsion and extracorporeal membrane oxygenation in the poisoned patient? Clin Toxicol 2015;53:145–150.


Levine M, Brooks DE, Franken A, Graham R. Delayed-onset seizure and cardiac arrest after amitriptyline

References
References

overdose, treated with intravenous lipid emulsion therapy. *Pediatrics* 2012;130:e432–8.


Rosenberg PH, Kalso EA, Tuominen MK, Lindén HB. Acute bupivacaine toxicity as a result of venous leakage under the tourniquet cuff during a Bier block. Anesthesiology 1983;58:95–98.


Scherrer V, Compere V, Loisel C, Dureuil B. Cardiac arrest from local anesthetic toxicity after a field


Smith SJM. EEG in neurological conditions other than epilepsy: when does it help, what does it add? *J Neurol Neurosurg Psychiatr* 2005;76 (Suppl. 2):i8–12.


References
APPENDIX

APPENDIX 1 QUESTIONNAIRE (TRANSLATED IN ENGLISH)

QUESTIONNAIRE REGARDING LOCAL ANAESTHETIC SYSTEMIC TOXICITY 2011–2013

<table>
<thead>
<tr>
<th>RESPONDER INFORMATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
</tr>
<tr>
<td>Phone number</td>
</tr>
<tr>
<td>Department</td>
</tr>
<tr>
<td>Email</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NUMBER OF LOCAL ANAESTHETIC PROCEDURES PERFORMED BY ANAESTHESIOLOGISTS DURING 2011–2013 (TOTAL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>For surgery and post-operative pain management</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Spinal anaesthesia</td>
</tr>
<tr>
<td>Epidural anaesthesia</td>
</tr>
<tr>
<td>CSE (combined spinal and epidural anaesthesia)</td>
</tr>
<tr>
<td>Regional block</td>
</tr>
<tr>
<td>Intravenous regional anaesthesia</td>
</tr>
<tr>
<td>Systemic lidocaine infusion</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>For obstetrics</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Spinal anaesthesia</td>
</tr>
<tr>
<td>Epidural anaesthesia</td>
</tr>
<tr>
<td>CSE (combined spinal and epidural anaesthesia)</td>
</tr>
<tr>
<td>Regional block</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>For management of chronic pain</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Spinal anaesthesia</td>
</tr>
<tr>
<td>Epidural anaesthesia</td>
</tr>
<tr>
<td>CSE (combined spinal and epidural anaesthesia)</td>
</tr>
<tr>
<td>Regional block</td>
</tr>
<tr>
<td>Intravenous regional anaesthesia</td>
</tr>
<tr>
<td>Systemic lidocaine infusion</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TREATMENT PROTOCOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our department has a treatment protocol for local anaesthetic systemic toxicity: Yes</td>
</tr>
<tr>
<td>If yes, intravenous lipid emulsion is part of the protocol: Yes</td>
</tr>
</tbody>
</table>
### LOCAL ANAESTHETIC SYSTEMIC TOXICITY IN THE DEPARTMENT 2011–2013

There has been a case/cases of local anaesthetic toxicity in our department during 2011–2013  

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>Most serious symptoms were central nervous system symptoms</th>
<th>Most serious symptoms were cardiac symptoms</th>
<th>Local anaesthetic toxicity was fatal</th>
</tr>
</thead>
</table>

If yes, intravenous lipid emulsion was used as a part of treatment of local anaesthetic toxicity  

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>Most serious symptoms were central nervous system symptoms</th>
<th>Most serious symptoms were cardiac symptoms</th>
<th>Local anaesthetic toxicity was fatal</th>
</tr>
</thead>
</table>