General Anesthesia Reversal (GAR) by Lipofundin

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Abstract

Two case reports of General Anesthesia Reversal (GAR) are described. It is the first time in the medical literature in which Lipofundin 20% successfully reversed general anesthesia. In a previous similar article it was mentioned only “Lipid Emulsion” without mentioning which one was used. There are at least 19 Lipid Emulsions:

1. CELEPID
2. ClinOleic
3. Deltalipid
4. ELOLIPID
5. LÍPIDOS
6. INTRALIPID
7. IVELIP
8. Lipidem
9. Lipofundin
10. Liposyn
11. Lipovenös
12. Nutriflex
13. SMOFLIPID
14. Omegaven
15. Kombilipid
16. Lipoplus
17. Optilipid
18. Salvilipid
19. STRUCTOLIPID

Even in the LAST protocol it is written “Lipid Emulsion (LE)” without being specific. Not all the Lipid Emulsions were created equal...not all lipid emulsions were tested on LAST cases. This article describes also the Lipid Emulsion-Mitochondrial Sink Effect as well as the Linoleic Acid (Main Component in Le)-Brain Mitochondria Interaction.

Keywords: Lipofundin, Intralipid, Lipid Emulsion, General Anesthesia Reversal (GAR).

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Case reports

Case 1

A 79 year old man with COPD, 1.60m height and 40kg weight who was diagnosed with stomach cancer, performed gastrectomy under total anesthesia and thoracic epidural analgesia (TEA). The patient was admitted into operating room and established intravenous access for 0.9% of NaCl infusion. Constant monitoring was ensured by ECG, heart rate, pulse oximetry (SpO2); non-invasive blood pressure measurements were taken every 2.5 minutes.

Thoracic epidural analgesia was conveniently performed in lateral position in the T7-T8 flowing 5cm in epidural space.

**Before induction:** Ketogenes 30mg and fentanyl 0.1mg, magnesium 20 mg/kg were administered. Induction of Anesthesia was induced with propofol 80 mg, fentanyl 0.1mg, and rocuronium 40mg. The trachea was intubated with TOF=0 and ventilation of the lungs was controlled. Anesthesia was maintained with total intravenous anesthesia by propofol 6mg/kg/h. Routine monitoring included end tidal carbon dioxide measurements.

**After induction:** 0.5 mg/kg of ketamine for IV injection, 4ml anaropin 0.5% was used by TEA during surgery.

Rocuronium 10 mg was additional when TOF>2. His heart rate and blood pressure did not vary significantly during 3 hours 25 minutes of the surgery.

Total anesthetic drugs during operation included 930mg propofol, 0.2mg fentanyl, 90mg rocuronium, ketogenic 30mg and 20mg anaropin (Ropivacaine hydrochloride) via thoracic epidural route.

Sugammadex 100 mg had been used for rocuronium block reversal after surgery had been finished. Tracheal tube was extubated upon 2 minutes after TOF=95% but he still did not respond to verbal order, could not open his mouth and his eyes within over 10 minutes during which other routine monitoring were good (respiratory rate 21l/min, SpO2=100%).

Lipofundin 20% was administered according to LAST protocol in this case with 100 ml bolus on the first time and intravenous infusion of 100 ml.

After the first bolus dose, this patient could open his eyes quickly. Then he could open his mouth and lift his head easily. The tracheal tube was extubated just after. He remained stable for the next 15 minutes and was discharged to patient's room.

**Note:** The first patient mentioned in this article had given his signed written permission to use his video clips taken in the recovery room for scientific purposes to all the scientific community all over the world.

Discussion

Recovery time and quality after general anaesthesia is important for patient safety. This study aimed to determine whether intravenous lipid emulsion could improve recovery profiles from isoflurane anaesthesia in adult patients undergoing laparoscopic cholecystectomy [1]. Sixty-six patients were enrolled. After anaesthesia induction, inspired isoflurane concentration was adjusted to maintain stable vital signs and the suitable conditions for operation. At the end of the operation, the isoflurane was discontinued, and either 2 ml/kg 30% lipid emulsions or 0.9% saline solution was administered intravenously. The time to eye opening, extubation and exit from the operation room was recorded, and the quality of recovery from anaesthesia was assessed. Sixty patients completed the study. The median time to eye opening and exit from the operation room was significantly shorter in the lipid emulsion group than in the saline group [15.5 (interquartile range 9.0) versus 20.0 (10.0) min., p=0.01; 19.5 (8.3) versus 23.6 (6.3) min., p=0.04, respectively], whereas the median time to extubation did not show any noticeable difference. The quality of recovery

Case 2

A 68 year old man with diabetes history (HbA1C=6.6%; glucose=4.98 m mol/l) 1.68m height and 68 kg was diagnosed with stomach cancer which indicated laparoscopic gastrectomy under total anesthesia and thoracic epidural analgesia (TEA). The patient was admitted into operating room and established intravenous access for 0.9% of NaCl infusion. Constant monitoring was ensured by ECG, heart rate, pulse oximetry (SpO2), non-invasive blood pressure measurements were taken every 2.5 minutes. Thoracic epidural analgesia was conveniently performed in the lying on the side position in the T7-T8, flowing 10cm in the epidural cavity. Anesthesia was induced with propofol 120mg, fentanyl 0.15mg and rocuronium 50mg. The trachea was intubated with TOF=0 and ventilation of the lungs was controlled. Anesthesia was maintained by sevoflurane in oxygen. Routine monitoring included end tidal carbon dioxide measurements. 5ml anaropin (Ropivacaine hydrochloride) 0.25% was used by TEA before incision. Rocuronium 1mg/ml was given by the intravenous route as well as 10ml/h Ketogenic 30mg and fentanyl 0.1 mg and ondansetron 4 mg were administered. His heart rate and blood pressure did not vary significantly during 5 hours 45 minutes of surgery. Total anesthetic drugs for this operation included 120mg propofol, 0.25 mg fentanyl, 115 mg rocuronium, ketogenic 30mg, 4 mg ondansetron, sevoflurane MAC, and 40 mg anaropin with TEA. Prostigmine 2mg and atropine 0.75mg had been used to reverse rocuronium blockade after his surgery was finished. In the next 8 minutes TOF=95% but he had no motion during the next 15 minutes while other routine monitoring was good. Lipofundin 20% was used according to the LAST protocol in this case with 100 ml bolus on the first time and intravenous infusion of 100 ml.

After the first bolus dose, this patient could open his eyes quickly. Then he could open his mouth and lift his head easily. The tracheal tube was extubated just after. He remained stable for the next 15 minutes and was discharged to patient's room.

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was better in the lipid emulsion group than that of the saline solution group with respect to drowsiness visual analogue scale score (p<0.01), Observer’s Assessment of Alertness/Sedation score (p<0.01), Mini-Mental State Examination score (p=0.04) and Modified Aldrete Post Anaesthesia Recovery score (p=0.03). No serious adverse events were observed during the study period. In conclusion, intravenous lipid emulsion may effectively improve the recovery time and quality from isoflurane anaesthesia for laparoscopic cholecystectomy [1].

Surgery puts a lot of stress on the body, and during recovery from surgery, the body uses a lot of energy to help with healing and getting stronger. Often, sugars are given before surgery to help give the body an energy boost. Lipid solutions can also be used as an energy source and are commonly used as supplements in patients needing long-term nutrition from an intravenous route (e.g., total parenteral nutrition) when they cannot eat by mouth for a medical reason. Intralipid, a solution of lipid molecules from soybeans and eggs, is commonly used for patients who need nutrition and energy supplements. The investigators wish to test whether giving Intralipid immediately after surgery can improve recovery from major surgery [2].

The perioperative events associated with major surgery trigger the body’s stress response, compromising optimal post-surgical recovery and outcomes. Moreover, traditional perioperative indications, such as restricting food up to two days before surgery, have been shown to be associated with poor outcomes following surgery. The Enhanced Recovery after Surgery (ERAS) protocol, initiated in the early 2000’s, aimed to address these problems. The ERAS protocol has recommendations for pre-, intra-, and post-operative stages, with the goal of modifying physiological and psychological responses to surgery. A key component of ERAS is perioperative nutrition, including avoidance of fasting before surgery and carbohydrate loading up to two hours pre-surgery. It is hypothesized that adequate nutrition and provision of an energy source in the perioperative period can attenuate the body’s stress response during surgery, thereby enabling faster and more successful recovery.

Early post-operative feeding is another recommendation of ERAS and is also intended to ensure optimal metabolic balance. Post-operative nutrition of ERAS patients generally involves oral feeding with energy-dense nutritional supplements in the days after surgery until the patient is ready for normal food intake. Lipids and carbohydrates form the bulk of these supplements, providing a readily available energy source. Lipid emulsions, such as Intralipid®, are used commonly in total parenteral nutrition and are also indicated as an energy source in patients for whom the usual intravenous fluid therapy would not be adequate. Lipid emulsions alone may therefore be helpful in enhancing recovery following major surgery, particularly in patients who are not good candidates for a strict ERAS protocol. With respect to short-term outcomes following surgery, Intralipid has been shown to modulate blood pressure by increasing systemic vascular resistance and cardiac output.

Only one study so far has evaluated post-surgery recovery outcomes following administration of lipid emulsion exclusively. The investigators found that patients receiving an intravenous infusion of lipid emulsion experienced better overall recovery and faster eye opening and exit from the operating room compared to patients receiving an infusion of 0.9% normal saline. While these results suggest that post-operative lipid delivery improves recovery, the study did not assess differences in basic vital signs between the two groups, and the subjects were limited to those undergoing laparoscopic cholecystectomy. In this study, the investigators will recruit patients undergoing any surgery of the abdomen and assess post-operative outcomes, including vital signs, following an intravenous bolus of Intralipid® 20% or 0.9% normal saline.

**Objective**

To demonstrate that patients receiving an intravenous bolus of Intralipid® 20% immediately following surgery experience improved post-surgical recovery compared to patients receiving an intravenous saline bolus.

**Hypothesis**

The investigators hypothesize that patients who receive an intravenous bolus of Intralipid® following major abdominal surgery will show more stable vital signs and faster discharge from the recovery room compared to patients receiving a saline bolus [2]. This study was done between May 2015 to April 2017. No article was published yet with its results.

**Lipid Emulsion-Mitochondrial Sink Effect**

Papadopoulou A et al. [3] hypothesized that by substituting a dye surrogate in place of local anesthetic, they could visually demonstrate dye sequestration by lipid emulsion that would be dependent on both dye lipophilicity and the amount of lipid emulsion used.

They selected 2 lipophilic dyes, acid blue 25 and Victoria blue, with log P values comparable to lidocaine and bupivacaine, respectively. Each dye solution was mixed with combinations of lipid emulsion and water to emulate “lipid rescue” treatment at dye concentrations equivalent to fatal, cardiotoxic, and neurotoxic local anesthetic plasma concentrations. The lipid emulsion volumes added to each dye solution emulated equivalent intravenous doses of 100, 500, and 900 mL of 20% Intralipid in a 75 kgs adult. After mixing the samples were separated into a lipid-rich supernatant and a lipid-poor subnatant by heparin flocculation. The subnatants were isolated, and their colours compared against a graduated dye concentration scale.

Lipid emulsion addition resulted in significant dye acquisition by the lipid compartment accompanied by a reduction in the colour intensity of the aqueous phase that could be readily observed. The greatest amount of sequestration occurred with the dye possessing the higher log P value and the greatest amount of lipid emulsion.

This study provides a visual demonstration of the lipid sink effect. It supports the theory that lipid emulsion may
reduce the amount of free drug present in plasma from concentrations associated with an invariably fatal outcome to those that are potentially survivable.

Local anesthetic (LA) intoxication with cardiovascular arrest is a potential fatal complication of regional anesthesia. Lipid resuscitation has been recommended for the treatment of LA-induced cardiac arrest. Aim of the study was to compare four different rescue regimens using epinephrine and/or lipid emulsion and vasopressin to treat cardiac arrest caused by bupivacaine intoxication [4].

Twenty-eight piglets were randomized into four groups (4×7), anesthetized with sevoflurane, intubated, and ventilated. Bupivacaine was infused with a syringe driver via central venous catheter at a rate of 1 mg·kg⁻¹·min⁻¹ until circulatory arrest. Bupivacaine infusion and sevoflurane were then stopped, chest compression was started, and the pigs were ventilated with 100% oxygen. After 1 min, epinephrine 10 μg·kg⁻¹ (group 1), Intralipid® 20% 4 ml·kg⁻¹ (group 2), epinephrine 10 μg·kg⁻¹+Intralipid® 4 ml·kg⁻¹ (group 3) or 2 IU vasopressin+Intralipid® 4 ml·kg⁻¹ (group 4) were administered. Secondary epinephrine doses were given after 5 min if required.

Survival was 71%, 29%, 86%, and 57% in groups 1, 2, 3, and 4. Return of spontaneous circulation was regained only by initial administration of epinephrine alone or in combination with Intralipid® (®). Piglets receiving the combination therapy survived without further epinephrine support. In contrast, in groups 2 and 4, return of spontaneous circulation was only achieved after secondary epinephrine rescue.

In cardiac arrest caused by bupivacaine intoxication, first-line rescue with epinephrine and epinephrine+Intralipid® more effective with regard to survival than Intralipid® alone and vasopressin+Intralipid® in this pig model [5].

Local anesthetic (LA) intoxication with severe hemodynamic compromise is a potential catastrophic event. Lipid resuscitation has been recommended for the treatment of LA-induced cardiac arrest. However, there are no data about effectiveness of Intralipid® for the treatment of severe cardiovascular compromise prior to cardiac arrest. Aim of this study was to compare effectiveness of epinephrine and Intralipid® for the treatment of severe Hemodynamic compromise owing to bupivacaine intoxication, anesthetized Piglets were with sevoflurane, intubated, and ventilated. Bupivacaine was infused with a syringe driver via a central venous catheter at a rate of 1 mg·kg⁻¹·min⁻¹ until invasively measured mean arterial pressure (MAP) dropped to 50% of the initial value. Bupivacaine infusion was then stopped, and epinephrine 3 μg·kg⁻¹ (group 1), Intralipid® 20% 2 ml·kg⁻¹ (group 2), or Intralipid® 20% 4 ml·kg⁻¹ (group 3) was immediately administered. Twenty-one piglets (3×7), were recorded. All animals in group 1 (100%) but only four of seven (57%) piglets in group 2 and group 3, respectively, survived. Normalization of hemodynamic parameters (HR, MAP) and ET (CO₂) was fastest in group 1 with all piglets achieving HR and MAP values, hemodynamic compromise owing to bupivacaine intoxication in piglets, first-line rescue with epinephrine was more effective than Intralipid® with regard to survival as well as normalization of hemodynamic parameters and ET (CO₂) [6].

Intravenous lipid emulsion (ILE) has been proposed as a rescue therapy for severe local anesthetic drugs toxicity, but experience is limited with other lipophilic drugs. An 18-year-old healthy woman was admitted 8 h after the voluntary ingestion of sustained-release diltiazem (3600 mg), with severe hypotension refractory to fluid therapy, calcium salts, and high-dose norepinephrine (6.66 μg/kg/min). Hyperinsulinemia Euglycemia therapy was initiated and shortly after was followed by a protocol of ILE (intralipid 20%, 1.5 ml/kg as bolus, followed by 0.25 ml/kg over 1h). The main finding attributed to ILE was an apparent rapid decrease in insulin resistance, despite a prolonged serum diltiazem elimination half-life. Diltiazem is a lipophilic cardio toxic drug, which could be sequestered in an expanded plasma lipid phase. The mechanism of action of ILE is not known, including its role in insulin resistance and myocardial metabolism in calcium-channel blocker poisoning [7].

**Linoleic Acid (Main Component in LE) - Brain Mitochondria Interaction**

Linoleic acid (LA; 18:2 n-6), the most abundant polyunsaturated fatty acid in the US diet, is a precursor to oxidized metabolites that have unknown roles in the brain. Here, we show that oxidized LA-derived metabolites accumulate in several rat brain regions during CO₂-induced ischemia and that LA-derived 13-hydroxyoctadecadienoic acid, but not LA, increase somatic paired-pulse facilitation in rat hippocampus by 80%, suggesting bioactivity. This study provides new evidence that LA participates in the response to ischemia-induced brain injury through oxidized metabolites that regulate neurotransmission. Targeting this pathway may be therapeutically relevant for ischemia related conditions such as stroke [8].

Long-chain polyunsaturated fatty acids like conjugated linoleic acids (CLA) are required for normal neural development and cognitive function and have been ascribed various beneficial functions. Recently, oral CLA also has been shown to increase testosterone (T) biosynthesis, which is known to diminish traumatic brain injury (TBI)-induced neuropathology and reduce deficits induced by stroke in adult rats. To test the impact of CLA on cognitive recovery following a TBI, 5-6 month old male Sprague Dawley rats received a focal injury (cortical impact; n=17) or Sham injury (craniectomy alone; n=12) and were injected with 25 mg/kg body weight of Clarinol® G-80 (80% CLA in safflower oil; n=16) or saline (n=13) every 48 hrs for 4 weeks. Sham surgery decreased baseline plasma progesterone (P4) by 64.2% (from 9.5 ± 3.4 ng/mL to 3.4 ± 0.5 ng/mL; p=0.068), T by 74.6% (from 5.9 ± 1.2 ng/mL to 1.5 ± 0.3 ng/mL; p<0.05), 11-deoxycorticosterone (11-DOC) by 37.5% (from 289.3 ± 42.0 ng/mL to 180.7 ± 3.3 ng/mL), and corticosterone by 50.8% (from 195.1 ± 22.4 ng/mL to 95.9 ± 2.2 ng/mL), post-surgery day 1. CCI injury induced similar declines in P4, T, 11-DOC and corticosterone (58.9%,
74.6%, 39.4% and 24.6%, respectively) by post-surgery day 1. These results suggest that both Sham surgery and CCI injury induce hypogonadism and hypo-adrenalism in adult male rats. CLA treatment did not reverse hypogonadism in Sham (P4: 2.5 ± 1.0 ng/mL; T: 0.9 ± 0.2 ng/mL) or CCI injured (P4: 2.2 ± 0.9 ng/mL; T: 1.0 ± 0.2 ng/mL, p<0.05) animals by post-injury day 29, but rapidly reversed by post injury day 1 the hypo-adrenalism in Sham (11-DOC: 372.6 ± 36.6 ng/mL; corticosterone: 202.6 ± 15.6 ng/mL) and CCI-injured (11-DOC: 384.2 ± 101.3 ng/mL; corticosterone: 234.6 ± 43.8 ng/mL) animals. In Sham surgery animals, CLA did not alter body weight, but did markedly increase latency to find the hidden Morris Water Maze platform (40.3 ± 13.0s) compared to saline treated Sham animals (8.8 ± 1.7s). In CCI injured animals, CLA did not alter CCI-induced body weight loss, CCI-induced cystic infarct size, or deficits in rotarod performance. However, like Sham animals, CLA injections exacerbated the latency of CCI-injured rats to find the hidden MWM platform (66.8 ± 10.6 s) compared to CCI injured rats treated with saline (30.7 ± 5.5 s, p<0.05). These results indicate that chronic treatment of CLA at a dose of 25 mg/kg body weight in adult male rats over 1-month 1) does not reverse creniactomy- and cranietomy + CCI induced hypogonadism, but does reverse cranietomy and cranietomy + CCI-induced hypo-adrenalism, 2) is detrimental to medium- and long-term spatial learning and memory in cranietomized uninjured rats, 3) limits cognitive recovery following a moderate-severe CCI injury, and 4) does not alter body weight [9].

Oxidative damage of membrane unsaturated fatty acids (PUFA) is thought to play a major role in mitochondrial dysfunction related to Parkinson’s disease (PD). The toxic products formed by PUFA oxidation inflict further damage on cellular components and contribute to neuronal degeneration. Here, we tested the hypothesis that isotopic enrichment, by de-uteration of the bi-salicylic sites most susceptible to oxidation in PUFA may provide at least partial protection against nigrostriatal injury in a mouse model of oxidative stress and cell death, the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model. Mice were fed a fat-free diet supplemented with saturated acids, oleic acid and essential PUFA: either normal, hydrogenated linoleic (LA, 18:2 n-6) and α-linolenic (ALA, 18:3 n-3) or deuterated 11,11-D2-LA and 11,11,14,14-D4-ALA in a ratio of 1:1 (to a total of 10% mass fat) for 6 days; each group was divided into two cohorts receiving either MPTP or saline and then continued on respective diets for 6 days. Brain homogenates from mice receiving deuterated PUFA (D-PUFA) vs. hydrogenated PUFA (H-PUFA) demonstrated a significant incorporation of deuterium as measured by isotope ratio mass-spectrometry. Following MPTP exposure, mice fed H-PUFA revealed 78.7% striatal dopamine (DA) depletion compared to a 46.8% reduction in the D-PUFA cohort (as compared to their respective saline-treated controls), indicating a significant improvement in DA concentration with D-PUFA. Similarly, higher levels of the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were detected in MPTP-exposure mice administered D-PUFA; however, saline-treated mice revealed no change in DA or DOPAC levels. Western blot analyses of tyrosine hydroxylase (TH) confirmed neuroprotection with D-PUFA, as striatal homogenates showed higher levels of TH immune-reactivity in D-PUFA (88.5% control) vs. H-PUFA (50.4% control) in the MPTP-treated cohorts. In the substantia nigra, a significant improvement was noted in the number of nigral dopaminergic neurons following MPTP exposure in the D-PUFA (79.5% control) vs. H-PUFA (58.8% control) mice using unbiased stereological cell counting. Taken together, these findings indicate that dietary isotopic reinforcement with D-PUFA partially protects against nigrostriatal damage from oxidative injury elicited by MPTP in mice [10].

Arachidonic acid (AA), 5,8,11,14-eicosatetraenoic acid is abundant, active and necessary in the human body. In the present study, we reported the neuroprotective effects and mechanism of arachidonic acid on hippocampal slices insulted by glutamate, NaN₃ or H₂O₂ in vitro. Different types of models of brain injury in vitro were developed by 1mM glutamate, 10mM NaN₃ or 2mM H₂O₂. After 30 min of pre-incubation with arachidonic acid or linoleic acid, hippocampal slices were subjected to glutamate, NaN₃ or H₂O₂ then the tissue activities were evaluated by using the 2,3,5-triphenyltetrazolium chloride method. Endogenous antioxidant enzymes activities (SOD, GSH-PX and catalase) in hippocampal slices were evaluated during the course of incubation. MK886 (5 microM; a non-competitive inhibitor of proliferator-activated receptor [PPAR] alpha), BADGE (bisphenol A diglycidyl ether; 100 microM; an antagonist of PPARgamma) and cycloheximide (CHX; 30 microM; an inhibitor of protein synthesis) were tested for their effects on the neuroprotection afforded by arachidonic acid. Population spikes were recorded in randomly selected hippocapal slices. Arachidonic acid (1-10 microM) dose dependently protected hippocampal slices from glutamate and H₂O₂ injury (P<0.01), and arachidonic acid (10 microM) can significantly improve the activities of Cu/Zn-SOD in hippocampal slices after 1h incubation. In addition, 10 microM arachidonic acid significantly increased the activity of Mn-SOD and catalase, and decreased the activities of Cu/Zn-SOD to control value after 3h incubation. These secondary changes of SOD during incubation can be reversed by indomethacine (10 microM; a nonspecific cyclooxygenase inhibitor) or AA 861 (20 microM; a 5- lipooxygenase inhibitor). Its neuroprotective effect was completely abolished by BADGE and CHX. These observations reveal that arachidonic acid can defend against oxidative stress by boosting the internal antioxidant system of hippocampal slices. Its neuroprotective effect may be mainly mediated by the activation of PPARgamma and synthesis of new protein in tissue [11].

Free fatty acid (FFA) concentrations in cerebrospinal fluid (CSF) are recognized as markers of brain damage in animal studies. There is, however, relatively little information regarding FFA concentrations in human CSF in normal and pathological conditions. The present study examined FFA concentrations in CSF from 15 patients with traumatic brain injury (TBI) and compared the data with values obtained from 73 contemporary controls. Concentrations of specific FFAs from TBI patients, obtained within 48 h of the insult were significantly greater than those
in the control group (arachidonic, docosahexaenoic and myristic, P<0.001; oleic, palmitic, P<0.01; linoleic, P<0.05). Higher concentrations of total polyunsaturated fatty acids (P<0.001) and of arachidonic, myristic and palmitic acids measured individually in CSF (P<0.01) obtained 1 week after the insult were associated with a worse outcome at the time of hospital discharge using the Glasgow Outcome Scale. This preliminary investigation suggests that CSF FFA concentrations may be useful as a predictive marker of outcome following TBI [12].

Elevated levels of free fatty acids (FFA) have been implicated in the pathogenesis of neuronal injury and death induced by cerebral ischemia. This study evaluated the effects of immune-suppressants agents, calcineurin inhibitors and blockade of endoplasmic reticulum (ER) calcium channels on free fatty acid formation and efflux in the ischemic/reperfused (I/R) rat brain. Changes in the extracellular levels of arachidonic, docosahexaenoic, linoleic, myristic, oleic four-vessel occlusion-elicted global cerebral ischemia were examined using a cortical cup technique. A 20-min period of ischemia elicited large increases in the efflux of all six FFAs, which were sustained during the 40 min of reperfusion. Cyclosporin A (CsA) and trifluoperazine, which reportedly inhibit the I/R elicited opening of a mitochondrial permeability transition (MPT) pore, were very effective in suppressing ischemia/reperfusion evoked release of all six FFAs. FK506, an immunosuppressant which does not directly affect the MPT, but is a calcineurin inhibitor, also suppressed the I/R-evoked efflux of FFAs, but less effectively than CsA. Rapamycin, a derivative of FK506 which does not inhibit calcineurin, did not suppress I/R-evoked FFA efflux. Gossypol, a structurally unrelated inhibitor of calcineurin, was also effective, significantly reducing the efflux of docosahexaenoic, arachidonic and oleic acids. As previous experiments had implicated elevated Ca\textsuperscript{2+} levels in the activation of phospholipases with FFA formation, agents affecting endoplasmic reticulum stores were also evaluated. Dantrolene, which blocks the ryanodine receptor (RyR) channel of the ER, significantly inhibited I/R-evoked release of docosahexaenoic, arachidonic, linoleic and oleic acids. Ryanodine, which can either accentuate or block Ca\textsuperscript{2+} release, significantly enhanced ischemia/reperfusion elicited efflux of linoleic acid, with non-significant increases in the efflux of myristic, arachidonic, palmitic and oleic acids. Xestospongion C, an inhibitor of the inositol triphosphate (IP3R) channel, failed to affect I/R-evoked FFA efflux. Thapsigargin, an inhibitor of the Ca2+-ATPase ER uptake pump, elicited significant elevations in the efflux of myristic, arachidonic and linoleic acids, in the absence of ischemia. Collectively, the data suggest an involvement of both ER and mitochondrial Ca2+ stores in the chain of events which lead to PLAA2 activation and FFA formation [13].

Brain extracellular levels of glutamate, aspartate, GABA and glycine increase rapidly following the onset of ischemia, remain at an elevated level during the ischemia, and then decline over 20-30 min following reperfusion. The elevated levels of the excitotoxic amino acids, glutamate and aspartate, are thought to contribute to ischemia-evoked neuronal injury and death. Calcium-evoked exocytotic release appears to account for the initial (1-2 min) efflux of neurotransmitter-type amino acids following the onset of ischemia, with non-vesicular release responsible for much of the subsequent efflux of these and other amino acids, including taurine and phospho-ethanolamine. Extracellular Ca\textsuperscript{2+} independent release is mediated, in part by Na\textsuperscript{+} dependent amino acid transporters in the plasma membrane operating in a reversed mode, and by the opening of swelling induced chloride channels, which allow the passage of amino acids down their concentration gradients. Experiments on cultured neurons and astrocytes have suggested that it is the astrocytes which make the primary contribution to this amino acid efflux. Inhibition of phospholipase A2 attenuates ischemia-evoked release of both amino and free fatty acids from the rat cerebral cortex indicating that this group of enzymes is involved in amino acid efflux, and also accounting for the consistent ischemia-evoked release of phosphoethanolamine. It is, therefore, possible that disruption of membrane integrity by phospholipases plays a role in amino acid release. Recovery of amino acid levels to pre ischemic levels requires their uptake by high affinity Na\textsuperscript{+} dependent transporters, operating in their normal mode, following restoration of energy metabolism, cell resting potentials and ionic gradients [14].

Free fatty acid (FFA) elevation in the brain has been shown to correlate with the severity of damage in ischemic injury. The etiology of this increase in FFA remains unclear and has been hypothesized to result from phospholipase activation. This study examines the effects of specific phospholipase inhibitors on FFA efflux during ischemia-reperfusion injury. A four-vessel occlusion model of cerebral ischemia was utilized to assess the effects of PLA2 and PLC inhibitors on FFA efflux from rat cerebral cortex. In addition, FFA efflux from non-ischemic cortices exposed to PLA2 and PLC was measured. Concentrations of arachidonic, docosahexaenoic, linoleic, myristic, oleic, and palmitic acids in cortical super fusates were determined using high performance liquid chromatography (HPLC). Exposure to the non-selective PLA2 inhibitor 4-bromophenylacyl bromide (BPB) significantly inhibited FFA efflux during ischemia-reperfusion injury (P<0.01 arachidonic, oleic and palmitic; P<0.05 all others); exposure to the PLC inhibitor U73122 had no observed effect. The effects of the Ca\textsuperscript{2+} dependent PLA2 inhibitor arachidonyl trifluoromethyl ketone (AACOCF3) mirrored the effects of BPB and led to reductions in all FFA levels (P<0.01 arachidonic, oleic and palmitic; P<0.05 all others). Exposure to the secretory PLA2 inhibitor 3-(3-acetamide-1-benzyl-2-ethyl-indol-5-oxy) propane sulfonic acid (LY311727) and to the Ca\textsuperscript{2+} independent PLA2 inhibitor bromoeno lactone (BEL) had only minimal effects on FFA efflux. Application of both PLA2 and PLC to non-ischemic cortices resulted in significant increases in efflux of all FFA (P<0.05). The study suggests that FFA efflux during ischemia-reperfusion injury is coupled to activation of Ca\textsuperscript{2+} dependent PLA2 and provides further evidence of the potential neuroprotective benefit of Ca\textsuperscript{2+} dependent PLA2 inhibitors in ischemia [15].

**Conclusion**

Lipofundin 20% can reverse general anesthesia within a few minutes from its intravenous bolus administration. More
clinical studies are advised to test other lipid emulsions as well for that purpose.

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