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Intralipid Treatment of Preeclampsia/Eclampsia?

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Abstract

Calcium, Aspirin, antihypertensive drugs and Magnesium sulfate are drug Interventions that are recommended for prevention or treatment of pre-eclampsia and eclampsia by WHO [20]. This article suggests a new intervention of using Intralipid (fat emulsion) as a sole treatment or combined with the current drugs.

Intralipid is an emerging treatment for local anesthetic systemic toxicity as well as for other types of intoxications and it is also suggested for treatment in certain diseases.

Intralipid treatment of Preeclamsia/Eclampsia is first suggested in the medical literature. Animal studies should be done in order to evaluate this new treatment modality.

Keywords: Intralipid, Preeclamsia, Eclampsia, Natural killer cells, Calcium, Mitochondria.

Pre-eclamsia

Pre-eclampsia (PE) is a disorder of pregnancy characterized by the onset of high blood pressure and often a significant amount of protein in the urine [1,4]. When it arises, the condition begins after 20 weeks of pregnancy [2,3]. In severe disease there may be red blood cell breakdown, a low blood platelet count, impaired liver function, kidney dysfunction, swelling, shortness of breath due to fluid in the lungs, or visual disturbances [2,3]. Pre-eclampsia increases the risk of poor outcomes for both the mother and the baby [3]. If left untreated, it may result in seizures at which point it is known as eclampsia [2].

Eclampsia

Eclampsia is the onset of seizures (convulsions) in a woman with pre-eclampsia. Onset may be before, during, or after delivery. Most often it is during the second half of pregnancy. The seizures are of the tonic-clonic type and typically last about a minute. Following the seizure there is typically either a period of confusion or coma. Complications include aspiration pneumonia, cerebral hemorrhage, kidney failure, and cardiac arrest. Pre-eclampsia and eclampsia are part of a larger group of conditions known as hypertensive disorders of pregnancy [5].

Intralipid

Pulmonary arterial hypertension (PAH) is characterized by pulmonary vascular remodeling leading to right ventricular (RV) hypertrophy and failure. Intralipid (ILP), a source of parenteral nutrition for patients, contains a-linolenic acid and soy-derived phytoestrogens that are

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protective for lungs and heart. We therefore, investigated the therapeutic potential of ILP in preventing and rescuing monocrotaline-induced PAH and RV dysfunction [6]. PAH was induced in male rats with monocrotaline (60 mg/kg). Rats then received daily ILP (1 mL of 20% ILP per day IP) from day 1 to day 30 for prevention protocol or from day 21 to day 30 for rescue protocol. Other monocrotaline-injected rats were left untreated to develop severe PAH by day 21 or RV failure by approximately day 30. Saline or ILP-treated rats served as controls. Significant increase in RV pressure and decrease in RV ejection fraction in the RV failure group resulted in high mortality. Therapy with ILP resulted in 100% survival and prevented PAH-induced RV failure by preserving RV pressure and RV ejection fraction and preventing RV hypertrophy and lung remodeling. In pre-existing severe PAH, ILP attenuated most lung and RV abnormalities. The beneficial effects of ILP in PAH seem to result from the interplay of various factors, among which preservation and/or stimulation of angiogenesis, suppression and/or reversal of inflammation, fibrosis and hypertrophy, in both lung and RV, appear to be major contributors. In conclusion, ILP not only prevents the development of PAH and RV failure but also rescues pre-existing severe PAH [6].

In vitro investigations have revealed the ability of intralipids to suppress natural killer (NK) cytotoxicity. Evidence from both animal and human studies suggests that intralipid administered intravenously may enhance implantation and maintenance of pregnancy when the patient has an abnormal NK cell level or function.

The aim of this study was to establish the duration and efficacy of Intralipids suppressive effect on NK cell functional activity.

Fifty patients with abnormal NK activity results (NKa) received intralipid 20% i.v. (9 mg/mL total blood volume -corresponds to 2 mL of intralipid 20% diluted in 250 mL saline; or 18 mg/mL - corresponds to 4 mL of intralipid 20% diluted in 250 mL saline) infusions and their NKa were tested periodically. The determination of NK cell function was performed by flow cytometry using K562 cells as targets.

Fifty women with abnormal NKa-testing received intralipid infusions. 39(78%) showed NKa suppression within the normal range the first week after infusion, 11 (22%), showed suppression, but still above the normal threshold. They received second infusion 2-3 weeks later. In 10, the Nka activity was normalized the following week. Four patients had three intralipid infusions in 2-week periods in between and after the third infusion, and all showed NKa normal activity. In 47 patients the suppressive effect of the Intralipid after the normalization of NKa lasted between 6 and 9 weeks, in two patients this benefit lasted 5 weeks, and in one patient the effect was 4 weeks.

Intralipid is effective in suppressing *in vivo* abnormal NK-cell functional activity. The results suggest that Intralipid can be used successfully as a therapeutic option to modulate abnormal NK activity in women with reproductive failure [7].

Intralipid emulsion (ILE) is a nutritional fatty acid supplementation that is emerging as a potential therapy for local anesthetic systemic toxicity and is also being considered as a therapy for other lipophilic medication intoxications. Isolated reports of pulmonary edema or severe lipemia exist as a complication of therapy.

A 26-year-old hypertensive, male, kidney transplant recipient presented to an outside emergency department (ED) after an intentional overdose of his medications (ie, amlodipine, metoprolol, lisinopril). At presentation, he had hypotension and bradycardia that was unresponsive to treatment with intravenous saline, calcium, glucagon, and vasopressors. After failure of conventional therapy, an initial bolus of ILE (20%) was given with some improvement in his heart rate, and the dose was repeated. A continuous intravenous infusion of ILE therapy was started. The patient deteriorated, with development of both acute respiratory and renal failure. Continuous venovenous hemofiltration (CVVHF) was attempted to remove volume and correct metabolic abnormalities. Lipemic blood was immediately observed in the CVVHF filter. After 15 min, the transmembrane pressures of the filter began to rise in the absence of observed clotting of the blood and the filter then became completely obstructed. An attempt was made to remove the lipid by plasmapheresis to restart CVVHF, but the patient continued to deteriorate despite maximal vasopressor support. The patient's family decided to withdraw care and the patient expired. WHY SHOULD AN EMERGENCY PHYSICIAN BE AWARE OF THIS?: Emergency physicians treat patients with toxic ingestions on a regular basis. Being aware of possible complications of experimental antidote therapy, like ILE, can improve the treatment approach and outcomes for these patients [8].

Pregnant women with the vascular complication of preeclampsia show altered lipid metabolism characterized by elevated circulating triglycerides and non-esterified free fatty acids. We have compared the effect of maternal plasma from women with and without preeclampsia on cultured vascular endothelial cells and determined whether these plasma-induced changes were reproduced with free fatty acid solutions of palmitic, oleic and linoleic acid, representative of circulating levels reported in preeclampsia [9].

Lipid accumulation was quantified by oil-red O staining, apoptosis by terminal dUTP nick-end labelling (TUNEL) and the measurement of mitochondrial redox capacity, and membrane potential recorded using MTT reduction and JC-1 accumulation for human umbilical vein endothelial cells (HUVECs) exposed to plasma and free fatty acids.

Lipid droplet accumulation was significantly increased in cultured HUVECs conditioned with maternal plasma from pregnancies with preeclampsia compared with normal uncomplicated controls. This increase was replicated following exposure to free fatty acids at the combined concentrations defined in preeclampsia. Plasma from these women also caused a significant decrease in mitochondrial dehydrogenase activity, a marked reduction in mitochondrial membrane potential and an increase in apoptosis compared with normal pregnancy. Again these effects were reproduced

using free fatty acids in combination at the levels previously associated with preeclampsia.

These findings support the concept of a circulating pathogenic factor for preeclampsia and highlight the possibility that this factor is not a single compound but perhaps the combined elevation of the free fatty acids palmitic, oleic and linoleic acid in the maternal circulation [9].

Recently, we showed that levels of circulating free fatty acids are increased in women who later develop pre-eclampsia long before the clinical onset of the disease [10]. Among the serum free fatty acids, oleic-, linoleic-, and palmitic acid were found to be increased by 37, 25 and 25%, respectively. In the present study we asked if these free fatty acids can interfere with endothelial cell functions. Cultured endothelial cells were exposed to linoleic-, oleic- and palmitic acid in concentrations ranging from 0.016 to 0.133 mumol ml-1, resulting in molar ratios of free fatty acids to albumin of 0.2-1.6. We found that among these fatty acids, linoleic acid reduced the thrombin-stimulated prostacyclin release by 30-60%, oleic acid by 10-30%, whereas palmitic acid had no effect. Endothelial cells incubated in presence of linoleic acid showed a concentration-dependent reduction in prostacyclin release in response to thrombin, and cells incubated with linoleic acid for up to 28 h, showed a reduced thrombin-induced prostacyclin release at every time point. Endothelial level of cGMP mainly reflected the synthesis of endothelium-derived relaxing factor/nitrogen monoxide (EDRF/NO), since blocking of the endogenous production of EDRF/NO with N-omega-nitro-L-arginine, resulted in about 90% reduction in cGMP-content of the endothelial cells. Incubation with linoleic acid reduced the endothelial cGMP level by 70%. Linoleic acid reduced the endothelial cells ability to inhibit platelet aggregation by 10-45%, (p = 0.0019). It was concluded that linoleic acid impedes the ability of the endothelial cells to produce prostacyclin and cGMP, and to inhibit platelet aggregation [10].

To determine the effect of low doses of linoleic acid and calcium on prostaglandin (PG) levels and the efficacy of this treatment in the prevention of preeclampsia.

In a randomized, double-blind, placebo-controlled study we treated 86 primigravidas with risk factors for preeclampsia (high biopsychosocial risk [above 3 points], positive roll-over test, and high mean blood pressure [above 85 mmHg)] with daily doses of either 450 mg linoleic acid and 600 mg calcium (n=43) or 450 mg starch and 600 mg lactose placebo (n=43) during the third trimester of pregnancy [11].

Four women in the experimental group (9.3%) developed preeclampsia compared with 16 (37.2%) controls (relative risk 0.25, 95% confidence interval 0.09, 0.69, P<0.001). The median serum levels of PGE2 after 4 weeks of treatment increased by 106% in the experimental group (P=0.03) and decreased by 33% in the control group (P=0.02). The median ratio between thromboxane B2 and PGE2 decreased by 40% in the experimental group (P=0.02) and increased by 18% in the control group (P=0.14). No significant differences were observed in the median ratio between thromboxane B2 and 6-keto PGF1alpha in either group. No serious maternal or neonatal side effects of treatment occurred in either group.

The administration of low daily doses of linoleic acid and calcium during the third trimester of pregnancy reduced the incidence of preeclampsia significantly in women at high risk, possibly by correcting the PGE2 levels [11].

Calcium

Pre-eclampsia and eclampsia are common causes of serious morbidity and death. Calcium supplementation may reduce the risk of pre-eclampsia and may help to prevent preterm birth.

To assess the effects of calcium supplementation during pregnancy on hypertensive disorders of pregnancy and related maternal and child outcomes.

We searched the Cochrane Pregnancy and Childbirth Group's Trials Register (28 March 2013) and contacted study authors for more data where possible [12]. We updated the search in May 2014 and added the results to the 'Awaiting Classification' section of the review.

Randomised controlled trials (RCTs) comparing high-dose (at least 1 g daily of calcium) or low-dose calcium supplementation during pregnancy with placebo or no calcium.

We assessed eligibility and trial quality, extracted and double-entered data. High-dose calcium supplementation (≥ 1 g/day). We included 14 studies in the review, however one study contributed no data. We included 13 high-quality studies in our meta-analyses (15,730 women). The average risk of high blood pressure (BP) was reduced with calcium supplementation compared with placebo (12 trials, 15,470 women: risk ratio (RR) 0.65, 95% confidence interval (CI) 0.53 to 0.81; I^2=74%). There was also a significant reduction in the risk of pre-eclampsia associated with calcium supplementation (13 trials, 15,730 women: RR 0.45, 95% CI 0.31 to 0.65; I^2=70%). The effect was greatest for women with low calcium diets (eight trials, 10,678 women: average RR 0.36, 95% CI 0.20 to 0.65; I^2=76%) and women at high risk of pre-eclampsia (five trials, 587 women: average RR 0.22, 95% CI 0.12 to 0.42; I^2=0%). These data should be interpreted with caution because of the possibility of small-study effect or publication bias. The composite outcome maternal death or serious morbidity was reduced (four trials, 9732 women; RR 0.80, 95% CI 0.65 to 0.97; I^2=0%). Maternal deaths were not significantly different (one trial of 8312 women: calcium group one death versus placebo group six deaths). There was an anomalous increase in the risk of HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome (two trials, 12,901 women: RR 2.67, 95% CI 1.05 to 6.82; I^2=0%) in the calcium group, however, the absolute number of events was low (16 versus six).The average risk of preterm birth was reduced in the calcium group (11 trials, 15,275 women: RR 0.76, 95% CI 0.60 to 0.97; I^2=60%) and amongst women at high risk of developing pre-eclampsia (four trials, 568 women: average RR 0.45, 95% CI 0.24 to 0.83; I^2=60%), but no significant reduction in neonatal high care admission. There was no

overall effect on the risk of stillbirth or infant death before discharge from hospital (11 trials 15,665 babies: RR 0.90, 95% CI 0.74 to 1.09; I^2=0%).One study showed a reduction in childhood systolic BP greater than 95th percentile among children exposed to calcium supplementation in utero (514 children: RR 0.59, 95% CI 0.39 to 0.91). In a subset of these children, dental caries at 12 years old was also reduced (195 children, RR 0.73, 95% CI 0.62 to 0.87). Low-dose calcium supplementation (< 1 g/day) We included 10 trials (2234 women) that evaluated low-dose supplementation with calcium alone (4) or in association with vitamin D (3), linoleic acid (2), or antioxidants (1). Most studies recruited women at high risk for pre-eclampsia, and were at high risk of bias, thus the results should be interpreted with caution. Supplementation with low doses of calcium significantly reduced the risk of pre-eclampsia (RR 0.38, 95% CI 0.28 to 0.52; I^2=0%). There was also a reduction in hypertension, low birth weight and neonatal intensive care unit admission.

Calcium supplementation (≥ 1 g/day) is associated with a significant reduction in the risk of pre-eclampsia, particularly for women with low calcium diets. The treatment effect may be overestimated due to small-study effects or publication bias. It also reduces preterm birth and the occurrence of the composite outcome 'maternal death or serious morbidity'. We considered these benefits to outweigh the increased risk of HELLP syndrome, which was small in absolute numbers. The World Health Organization recommends calcium 1.5 g to 2 g daily for pregnant women with low dietary calcium intake. The limited evidence on low-dose calcium supplementation suggests a reduction in pre-eclampsia, but needs to be confirmed by larger, high-quality trials. Pending such results, in settings of low dietary calcium where high-dose supplementation is not feasible, the option of lower-dose supplements (500 to 600 mg/day) might be considered in preference to no supplementation [12].

A large percentage (16% of maternal mortality in developed countries, compared to 9% in developing countries), is due to hypertensive disorders in pregnancy. The etiology of preeclampsia remains unknown, with poorly understood pathophysiology. Magnesium and calcium play an important role in vascular smooth muscle function and therefore a possible role in the development of pre-eclampsia.

We aimed to compare serum magnesium and total calcium levels of preeclamptic and normal pregnant women at the Korle-Bu Teaching Hospital in Ghana [13].

A comparative cross-sectional study involving 30 normal pregnant and 30 preeclamptic women with >30 weeks gestation and aged 18-35 years, was conducted at the Korle-Bu Teaching Hospital. Magnesium and calcium were determined using a flame atomic absorption spectrometer.

Mean serum magnesium and total calcium levels in preeclamptic women were 0.70 ± 0.15 and 2.13 ± 0.30 mmol/L, respectively. Mean serum magnesium and total calcium levels in normal pregnant women were 0.76 ± 0.14 and 2.13 ± 0.35 mmol/L, respectively. There was a statistically nonsignificant difference in serum magnesium and total calcium in preeclamptic women compared to normal pregnant women, with p-values of 0.092 and 0.972, respectively.

Serum magnesium and total calcium, therefore, seem not to differ in preeclamptic women compared to normal pregnant women in Ghana [13].

Preeclampsia is a serious medical complication during pregnancy. In response to an increasing number of preeclamptic cases and scarcity of data concerning the interrelation between trace element levels and preeclampsia, we carried out a hospital-based case-control study in Riyadh, Saudi Arabia to study the correlation between levels of serum trace elements and risk of preeclampsia [14]. One hundred and twenty pregnant women were enrolled in this study and divided into three groups of 40 each-Control group, HR group (women at high risk of pre-eclampsia) and PET group (Pre-eclampsia group). Serum trace element levels were estimated by inductively coupled plasma optical emission spectrophotometer. The analysis found that mean values of Ca, Mg and Zn were 90.08 ± 6.38, 19.33 ± 3.32 and 1.30 ± 0.83 mg/L respectively in normotensive control and 77.85 ± 4.47, 15.44 ± 1.43 and 0.98 ± 0.63 mg/L respectively in the HR group. The mean values of Ca, Mg and Zn in the preeclamptic group were 70.37 ± 4.66, 13.58 ± 1.98 and 0.67 ± 0.59 mg/L, respectively. Interelement analysis reflected a negative correlation between Ca and Mg and between Mg and Zn whereas positive correlation between Ca and Zn in pre-eclamptic women. However the correlation was not statistically significant. In conclusion, our study suggests that decreased levels of these trace elements in serum may act as predisposing factors in pathogenesis of Pre-eclampsia [14].

Lipid Emulsion Effects on Mitochondria and Intracellular Calcium

Local anesthetic toxicity is thought to be mediated partly by inhibition of cardiac mitochondrial function. Intravenous (i.v.) lipid emulsion may overcome this energy depletion, but doses larger than currently recommended may be needed for rescue effect. In this randomized study with anesthetized pigs, we compared the effect of a large dose, 4 mL/kg, of i.v. 20% Intralipid® (n=7) with Ringer's acetate (n=6) on cardiovascular recovery after a cardiotoxic dose of bupivacaine [15]. We also examined mitochondrial respiratory function in myocardial cell homogenates analyzed promptly after needle biopsies from the animals. Bupivacaine plasma concentrations were quantified from plasma samples. Arterial blood pressure recovered faster and systemic vascular resistance rose more rapidly after Intralipid than Ringer's acetate administration ($p<0.0001$), but Intralipid did not increase cardiac index or left ventricular ejection fraction. The lipid-based mitochondrial respiration was stimulated by approximately 30% after Intralipid ($p<0.05$) but unaffected by Ringer's acetate. The mean (standard deviation) area under the concentration-time curve (AUC) of total bupivacaine was greater after Intralipid (105.2 (13.6) mg·min/L) than after Ringer's acetate (88.1 (7.1) mg·min/L) ($p=0.019$). After Intralipid, the AUC of the lipid-un-entrapped bupivacaine portion (97.0 (14.5)

mg·min/L) was 8% lower than that of total bupivacaine ($p<0.0001$). To conclude, 4 mL/kg of Intralipid expedited cardiovascular recovery from bupivacaine cardiotoxicity mainly by increasing systemic vascular resistance. The increased myocardial mitochondrial respiration and bupivacaine entrapment after Intralipid did not improve cardiac function [15-20].

Lipid emulsions have been used to treat various drug toxicities and for total parenteral nutrition therapy. Their usefulness has also been confirmed in patients with local anesthetic-induced cardiac toxicity. The purpose of this study was to measure the hemodynamic and composition effects of lipid emulsions and to elucidate the mechanism associated with changes in intracellular calcium levels in myocardiocytes.

We measured hemodynamic effects using a digital analysis system after Intralipid® and Lipofundin® MCT/LCT were infused into hearts hanging in a Langendorff perfusion system. We measured the effects of the lipid emulsions on intracellular calcium levels in H9c2 cells by confocal microscopy [20].

Infusion of Lipofundin® MCT/LCT 20% (1 ml/kg) resulted in a significant increase in left ventricular systolic pressure compared to that after infusing modified Krebs-Henseleit solution (1 ml/kg) ($P=0.003$, 95% confidence interval [CI], 2.4-12.5). Lipofundin® MCT/LCT 20% had a more positive inotropic effect than that of Intralipid® 20% ($P=0.009$, 95% CI, 1.4-11.6). Both lipid emulsion treatments increased intracellular calcium levels. Lipofundin® MCT/LCT (0.01%) increased intracellular calcium level more than that of 0.01% Intralipid® ($P<0.05$, 95% CI, 0.0-1.9).

These two lipid emulsions had different inotropic effects depending on their triglyceride component. The inotropic effect of lipid emulsions could be related with intracellular calcium level [16].

Accidental intravascular or high-dose injection of local anesthetics (LA) can result in serious, potentially life-threatening complications. Indeed, adequate supportive measures and the administration of lipid emulsions are required in such complications. The study's objectives were threefold: (i) evaluate the myocardial toxicity of levobupivacaine when administered intravenously; (ii) investigate levobupivacaine toxicity on cardiomyocytes mitochondrial functions and cellular structure; (iii) assess the protective effects of a lipid emulsion in the presence or absence of myocardial ischemia. Domestic pigs randomized into two groups of 24 animals each, with either preserved coronary circulation or experimental myocardial ischemia. Six animals from each group received either: (i) single IV injection of saline, (ii) lipid emulsion (Intralipid(®)), (iii) levobupivacaine, (iv) combination levobupivacaine-Intralipid(®) . Serially measured endpoints included: heart rate, duration of the monophasic action potentials (dMAP), mean arterial pressure, and peak of the time derivative of left ventricular pressure (LV dP/dtmax). In addition, the following cardiomyocytes mitochondrial functions were measured: reactive oxygen species (ROS) production, oxidative phosphorylation, and calcium retention capacity (CRC) as well as the consequences of ROS production on lipids, proteins, and DNA. IV injection of levobupivacaine induced sinus bradycardia and reduced dMAP and LV dP/dtmax . At the mitochondrial level, oxygen consumption and CRC were decreased. In contrast, ROS production was increased leading to enhanced lipid peroxidation and structural alterations of proteins and DNA. Myocardial ischemia was associated with global worsening of all changes. Intralipid(®) quickly improved haemodynamics. However, beneficial effects of Intralipid(®) were less clear after myocardial ischemia [17].

Cocaine intoxication leads to over 500,000 emergency department visits annually in the United States and ethanol cointoxication occurs in 34% of those cases. Cardiotoxicity is an ominous complication of cocaine and cocaethylene overdose for which no specific antidote exists. Because infusion of lipid emulsion (Intralipid) can treat lipophilic local anesthetic toxicity and cocaine is an amphipathic local anesthetic, the authors tested whether lipid emulsion could attenuate cocaine cardiotoxicity *in vivo* [18]. The effects of lipid emulsion were compared with the metabolically inert sulfobutylether-β-cyclodextrin (SBE-β-CD; Captisol) in an isolated heart model of cocaine and cocaethylene toxicity to determine if capture alone could exert similar benefit as lipid emulsion, which exhibits multimodal effects. The authors then tested if cocaine and cocaethylene, like bupivacaine, inhibit lipid-based metabolism in isolated cardiac mitochondria.

For whole animal experiments, Sprague-Dawley rats were anesthetized, instrumented, and pretreated with lipid emulsion followed by a continuous infusion of cocaine to assess time of onset of cocaine toxicity. For *ex vivo* experiments, rat hearts were placed onto a nonrecirculating Langendorff system perfused with Krebs-Henseleit solution. Heart rate, left ventricle maximum developed pressure (LVdevP), left ventricle diastolic pressure, maximum rate of contraction (+dP/dtmax), maximum rate of relaxation (-dP/dtmax), rate-pressure product (RPP=heart rate×LVdevP), and line pressure were monitored continuously during the experiment. A dose response to cocaine (10, 30, 50, and 100 μmol/L) and cocaethylene (10, 30, and 50 μmol/L) was generated in the absence or presence of either 0.25% lipid emulsion or SBE-β-CD. Substrate-specific rates of oxygen consumption were measured in interfibrillar cardiac mitochondria in the presence of cocaine, cocaethylene, ecgonine, and benzoylecgonine.

Treatment with lipid emulsion delayed onset of hypotension (140 seconds vs. 279 seconds; $p=0.008$) and asystole (369 seconds vs. 607 seconds; $p=0.02$) in whole animals. Cocaine and cocaethylene induced dose-dependent decreases in RPP, +dP/dtmax, and -dP/dtmax abs ($p<0.0001$) in Langendorff hearts; line pressure was increased by cocaine and cocaethylene infusion, but not altered by treatment. Lipid emulsion attenuated cocaine- and cocaethylene-induced cardiac depression. SBE-β-CD alone evoked a mild cardio depressant effect ($p<0.0001$) but attenuated further cocaine- and cocaethylene-induced decrements in cardiac

contractility at high concentrations of drug (100 μmol/L; $p<0.001$). Finally, both cocaine and cocaethylene, but not ecgonine and benzoylecgonine, inhibited lipid-dependent mitochondrial respiration by blocking carnitine exchange ($p<0.05$).

A commercially available lipid emulsion was able to delay progression of cocaine cardiac toxicity *in vivo*. Further, it improved acute cocaine- and cocaethylene- induced cardiac toxicity in rat isolated heart while SBE-β-CD was effective only at the highest cocaine concentration. Further, both cocaine and cocaethylene inhibited lipid-dependent mitochondrial respiration. Collectively, this suggests that scavenging-independent effects of lipid emulsion may contribute to reversal of acute cocaine and cocaethylene cardiotoxicity, and the beneficial effects may involve mitochondrial lipid processing [21].

We hypothesized that acute lipid-induced insulin resistance would be attenuated in high-oxidative muscle of lean trained (LT) endurance athletes due to their enhanced metabolic flexibility and mitochondrial capacity [22]. Lean sedentary (LS), obese sedentary (OS), and LT participants completed two hyperinsulinemic euglycemic clamp studies with and without (glycerol control) the coinfusion of Intralipid. Metabolic flexibility was measured by indirect calorimetry as the oxidation of fatty acids and glucose during fasted and insulin-stimulated conditions, the latter with and without lipid oversupply. Muscle biopsies were obtained for mitochondrial and insulin-signaling studies. During hyperinsulinemia without lipid, glucose infusion rate (GIR) was lowest in OS due to lower rates of nonoxidative glucose disposal (NOGD), whereas state 4 respiration was increased in all groups. Lipid infusion reduced GIR similarly in all subjects and reduced state 4 respiration. However, in LT subjects, fat oxidation was higher with lipid oversupply, and although glucose oxidation was reduced, NOGD was better preserved compared with LS and OS subjects. Mitochondrial performance was positively associated with better NOGD and insulin sensitivity in both conditions. We conclude that enhanced mitochondrial performance with exercise is related to better metabolic flexibility and insulin sensitivity in response to lipid overload [19].

The Theory is Everything

There is evidence that Eclampsia has a genetic origin. It is more common in pregnancies of children with Trisomy 13. Human leukocyte antigen (HLA) allosensitization is associated with a high rate of PET (pre-eclamptic toxaemia). It has been postulated that if the mother has an HLA-DR antigen allogeneic to the foetus, the maternal HLA cross the placenta to allosensitize the foetus. As a reaction, the mother produces immune complexes as well as immune active cytokines which cause events leading to PET. An abnormal placentation or abnormal placentation process is considered to be associated with or cause PET.

Many other factors and findings are thought to cause or accompany PET, such as: damage of the endothelial cells, increased placental cyclooxygenase activity and thromboxane production, lipid peroxidation due to free radicals with unsaturated fatty acids, release of tumor necrotizing factor (TNF) due to low antioxidant activity, damage to the endothelial cells (an injury of the endothelial cells disturbs the balance between coagulation and thrombocytes activation), elevated levels of asymmetric dimethylarginine. Elevated platelet thromboxane synthesis has been associated with PET, therefore a routine treatment with aspirin is usually recommended.

Elevated levels of certain markers like alpha feto protein (AFP) or cellular fibronectin (it was suggested that a high level of maternal fibronectin receptors might predict the onset of PET as well as abruptio placenta), autoantibodies agonistic to the angiotensin II type 1 (AT_1) receptor, von Willebrand factor as well as laminin or PP13, to name but a few, were found to be associated with PET and are used to predict the appearance of Eclampsia later in the pregnancy. A mean arterial pressure of 85-90mm Hg in the second trimester happens to be a predictor of pregnancy-induced hypertension.

Besides the mentioned markers for the prediction of toxaemia, the Doppler analysis yields reliable information concerning the vascular resistance index in the uterine arteries and is considered today to be an early marker for failure in perfusion associated with PET.

All the above-mentioned factors are evidence-based and definitely associated with the predisposition or clinical appearance of toxaemia. However, at the end of the 19th and beginning of the 20th centuries, the pointillist painters like George Seurat and Paul Signac composed their paintings by points of colour. Standing next to the picture reveals just points. However, taking a distance gives the impression of a coherent picture. It seems that PET, which is also called the disease of theories, has a similar fate. Doubtlessly, most observations are correct. Yet apparently most researchers stand too close to the picture, seeing just certain points. A new angle must be taken which will allow seeing the picture as a whole.

Natural Killer Cells

Natural killer (NK) cells constitute our bodies' frontline defense system, guarding against tumors and launching attacks against infections. The activities of NK cells are regulated by the interaction of various receptors expressed on their surfaces with cell surface ligands. While the role of NK cells in controlling tumor activity is relatively clear, the fact that they are also linked to various other disease conditions is now being highlighted. Here, we present an overview of the role of NK cells during normal body state as well as under diseased state. We discuss the possible utilization of these powerful cells as immunotherapeutic agents in combating diseases such as asthma, autoimmune diseases, and HIV-AIDS [21].

Human natural killer (NK) cells have distinct functions as NK (tolerant) , NK (cytotoxic) and NK (regulatory) cells and can be divided into different subsets based on the relative expression of the surface markers CD27 and CD11b. $CD27^+$ NK cells, which are abundant cytokine producers, are numerically in the minority in human peripheral blood

but constitute the large population of NK cells in cord blood, spleen, tonsil and decidua tissues. Recent data suggest that these NK cells may have immunoregulatory properties under certain conditions [22].

Natural killer (NK) cells recognize deranged cells that display stress receptors or loss of major histocompatibility complex (MHC) class I. During development, NK cells become "licensed" only after they encounter cognate human leukocyte antigen (HLA) class I, leading to the acquisition of effector function. NK cells can be exploited for cancer therapy in several ways. These include targeting with monoclonal antibodies alone or combined with *ex vivo* and *in vivo* NK cell activation to facilitate adoptive immunotherapy using donor-derived NK cell products to induce graft-vs-tumor effects. In the adoptive transfer setting, persistence and *in vivo* expansion requires lympho depleting chemotherapy to prevent rejection and provide homeostatic cytokines (such as IL-15) that activate NK cells. IL-15 has the advantage of avoiding regulatory T-cell expansion. Clinical applications are currently being tested. To enhance *in vivo* expansion, IL-2 has been used at low doses. However, low dose administration also leads to the stimulation of regulatory T cells. Monoclonal antibodies and bispecific killer engagers (BiKEs) may enhance specificity by targeting CD16 on NK cells to tumor antigens. Inhibition of CD16 shedding may also promote enhanced cytotoxicity. Future strategies include exploiting favourable donor immunogenetics or *ex vivo* expansion of NK cells from blood, progenitors, or pluripotent cells. Comparative clinical trials are needed to test these approaches [23].

The regulation of uterine and peripheral blood natural killer (NK) cells has been associated with problems related to reproductive immunology such as recurrent pregnancy loss (RPL), implantation failure or preeclampsia. NKp46, one of the natural cytotoxicity receptors (NCRs), is a unique marker that functions in NK cell cytotoxicity and cytokine production. Expression of NKp46 on NK cells is lower in women with recurrent pregnancy loss and pregnancy-induced hypertension. Moreover, expression of NKp46 on peritoneal fluid NK cells is lower in women with pelvic endometriosis. Therefore, evaluation of NKp46 on peripheral blood NK cells may provide a means of screening for reproductive abnormalities. Recently, a new type of NK cell, the NK22 cell, has been reported. This cell may be a regulator not only of the mucosal barrier but also of reproduction. For women with RPL showing abnormal uterine and/or peripheral blood NK cells, both intravenous immunoglobulin treatment and intralipid treatment have been reported. The effects of these treatments are still controversial, and further studies are needed in order to clarify their true impact [24].

The regulation of uterine and circulating peripheral blood natural killer (NK) cells has been associated with reproductive conditions including recurrent pregnancy loss (RPL), implantation failure and preeclampsia. Natural cytotoxicity receptors (NCRs) are unique markers that regulate NK cell cytotoxicity and cytokine production. The role of NCRs in reproductive events has not yet been fully characterized. There is an NK1 (Type 1) shift in peripheral blood NK cells in non-pregnant women prone to RPL and implantation failure. The different profile of NCR expression in endometrial or aborted decidual NK cells suggests the presence of abnormal regulation of NK cells in women with reproductive failure. Women with a history of RPL and preeclampsia carry immunological abnormalities of NCRs on peripheral blood NK cells during pregnancy. Evaluation of NKp46 on peripheral blood NK cells may be applicable for the prediction of preeclampsia. The lower expression of NKp46(+) NK cells in women with preeclampsia may account for the higher production of NK1 cytokines - known as the NK1 shift - in pregnant women with pre-eclampsia [25].

Natural cytotoxicity receptors (NCR) are unique markers that regulate natural killer (NK) cell cytotoxicity and cytokine production. In this study, we investigated the expression of NCR (NKp46, NKp44, and NKp30) and cytokine production in NK cells derived from the uterine endometrium of women with recurrent pregnancy loss (RPL). We also investigated the expression of NCR in peripheral blood NK cells in pregnant women with and without a history of RPL [26].

The expression of NCR (NKp46, NKp44, and NKp30) in NK cells ($CD56^{dim}$ and $CD56^{bright}$) in the uterine endometrium was analyzed using 3-color flow cytometry. Cytokine (tumor necrosis factor-α and interferon-γ) production was also analyzed. NK cells from the mid-secretory endometrium of 28 women with RPL, 34 women with implantation failure, and 74 controls were collected and mechanically dispersed using a tissue grinder. The expression of NCR in peripheral blood NK cells from pregnant women with (n=17) and without (n=91) a history of RPL was analyzed.

The percentages of $NKp46^{+}$ NK cells were significantly lower in both women with RPL and pregnant women with a history of RPL. The percentages of tumor necrosis factor-α- and/or interferon-γ-producing uterine endometrial NK cells were significantly lower in women with RPL compared with controls.

The changes in NCR expression and cytokine production, especially decreased NKp46 expression in endometrial NK cells, suggests the presence of abnormal NK cell regulation in women with reproductive failures [26].

Natural Killer Cell Memory

Natural killer (NK) cells have historically been considered short-lived cytolytic cells that can rapidly respond against pathogens and tumors in an antigen-independent manner and then undergo cell death. Recently, however, NK cells have been shown to possess traits of adaptive immunity and can acquire immunological memory in a manner similar to that of T and B cells [27].

Immunological memory has traditionally been regarded as a unique feature of the adaptive immune response, mediated in an antigen-specific manner by T and B lymphocytes. All other hematopoietic cells, including natural killer (NK) cells, are classified as innate immune cells, which have been considered short-lived but can respond rapidly

against pathogens in a manner not thought to be driven by antigen. Interestingly, NK cells have recently been shown to survive long term after antigen exposure and subsequently mediate antigen-specific recall responses [28].

Natural killer (NK) cells are generally considered to be part of the innate immune system. Over the past few years, however, evidence has accumulated suggesting that NK cells have certain features that are characteristic of the adaptive immune system. NK cells reportedly respond in an antigen-specific manner to a variety of small molecules and certain viruses, and mediate enhanced responses to these antigens upon secondary exposure. In infections with mouse cytomegalovirus (MCMV), MCMV-specific NK cells undergo clonal expansion, and display increased effector function after the resolution of the infection. In addition, inflammatory conditions resulting from exposure to certain cytokines seem to promote prolonged effector function in NK cells in an antigen-non-specific fashion. Taken together, these studies reveal new aspects of NK biology, and suggest that NK cells, like T and B cells, may carry out memory responses and may also exhibit greater capacity to distinguish antigens than was previously recognized [29].

Viral infections continuously challenge and shape our immune system. Due to their fine antigen recognition ability, adaptive lymphocytes protect against pathogen reencounter by generating specific immunological memory. Innate cells such as macrophages also adapt to pathogen challenge and mount resistance to reinfection, a phenomenon termed trained immunity. As part of the innate immunity, natural killer (NK) cells can display rapid effector functions and play a crucial role in the control of viral infections, especially by the β-herpesvirus cytomegalovirus (CMV). CMV activates the NK-cell pool by inducing proinflammatory signals, which prime NK cells, paralleling macrophage training. In addition, CMV dramatically shapes the NK-cell repertoire due to its ability to trigger specific NK cell-activating receptors and enables the expansion and persistence of a specific NK-cell subset displaying adaptive and memory features [30].

The functions of Natural Killer (NK) cells are regulated by a highly redundant set of germline-encoded surface receptors that can inhibit or activate NK cell activities. NK cells can be activated by cytokines or through the interaction with transformed or infected cells. This typically results in the production of cytokines, chemokines, and the induction of cellular cytotoxicity. However, the reactivity of NK cells is modulated on various levels and shaped by processes such as development, education, priming, exposure to antigens and cytokines, and the formation of memory-like phenotypes [31].

Immunological memory can be defined as a quantitatively and qualitatively enhanced immune response upon rechallenge. For natural killer(NK) cells, two main types of memory exist. First, similarly to T cells and B cells, NK cells can exert immunological memory after encounters with stimuli such as haptens or viruses, resulting in the generation of antigen-specific memory NK cells. Second, NK cells can remember inflammatory cytokine milieus that imprint long-lasting non-antigen-specific NK cell effector function. The basic concepts derived from studying NK cell memory provide new insights about innate immunity and could lead to novel strategies to improve treatments for infectious diseases and cancer [32].

Intralipid vs. Natural Killer Cells

In vitro investigations have revealed the ability of intralipids to suppress natural killer (NK) cytotoxicity. Evidence from both animal and human studies suggests that intralipid administered intravenously may enhance implantation and maintenance of pregnancy when the patient has an abnormal NK cell level or function.

The aim of this study was to establish the duration and efficacy of Intralipids suppressive effect on NK cell functional activity.

Fifty patients with abnormal NK activity results (NKa) received intralipid 20% i.v. (9mg/mL total blood volume -corresponds to 2 mL of intralipid 20% diluted in 250 mL saline; or 18 mg/mL - corresponds to 4 mL of intralipid 20% diluted in 250 mL saline) infusions and their NKa were tested periodically. The determination of NK cell function was performed by flow cytometry using K562 cells as targets.

Fifty women with abnormal NKa-testing received intralipid infusions. 39 (78%) showed NKa suppression within the normal range the first week after infusion, 11 (22%), showed suppression, but still above the normal threshold. They received second infusion 2-3 weeks later. In 10, the Nka activity was normalized the following week. Four patients had three intralipid infusions in 2-week periods in between and after the third infusion, and all showed NKa normal activity. In 47 patients the suppressive effect of the Intralipid after the normalization of NKa lasted between 6 and 9 weeks, in two patients this benefit lasted 5 weeks, and in one patient the effect was 4 weeks.

Intralipid is effective in suppressing *in vivo* abnormal NK-cell functional activity. The results suggest that Intralipid can be used successfully as a therapeutic option to modulate abnormal NK activity in women with reproductive failure [33].

To investigate the efficacy of intralipid supplementation in women with recurrent spontaneous abortion (RSA) and elevated natural killer cell activity undergoing *in vitro* fertilization/intracytoplasmic sperm injection.

Between February 10, 2013, and April 30, 2015, a double-blind randomized controlled study was conducted at a center in Egypt. Women with unexplained secondary infertility, RSA, and elevated levels of natural killer cells (>12%) were enrolled and randomly assigned to receive intralipid (2mL diluted at 20% in 250mL saline) or saline (250mL) infusion on the day of oocyte retrieval using random numbers and sealed envelopes. Patients and attending physicians were masked to group assignment. The infusions were repeated within 1week of a positive pregnancy test and then every 2 weeks until the end of the first trimester. The primary outcome was chemical pregnancy 14 days after embryo transfer. Analyses were by intention-to-treat.

Overall, 296 women were enrolled. Chemical pregnancy

was recorded for 84 (58.3%) of 144 women in the intralipid group and 76 (50.0%) of 152 in the control group (P=0.129).

Intralipid supplementation did not increase frequency of chemical pregnancy. However, findings related to ongoing pregnancy and live birth should be investigated further [34].

To evaluate the efficacy of intralipid intravenous infusion in achieving a live pregnancy following IVF--embryo transfer in women of advanced reproductive age (40-42 years). A matched control was performed. Women aged 40-42 with a previous history of miscarriage or who failed to conceive despite previous embryo transfer who entered an IVF program were offered intravenous intralipid therapy (four ml of 20% liposyn II in 100 ml normal saline over one hour) during the mid-follicular phase. Clinical pregnancy rates (eight weeks with viable gestation) and live delivered pregnancy rates were then determined and compared.

The results were evaluated after ten matched cycles. There were no clinical pregnancies in those receiving intralipid vs. a 40% clinical and a 30% live delivered pregnancy rate in the untreated controls (p=0.087, Fisher's exact test). The study was terminated because of these preliminary data.

In the test tube, adding intralipid to natural killer cells can inhibit their cytolytic action. However, the use of intravenous intralipid to suppress natural killer cell activity does not seem to improve the chance of a live delivery in women aged 40-42 years with a previous history of miscarriage. In fact, this therapy may actually be detrimental in this age group. Since efficacy of this therapy was not found in a group of advanced reproductive age, it is not clear why this should be effective for a younger population. A controlled study for the younger group is needed. Perhaps such a study could be limited to only those with miscarriage rather than also concluding failure to conceive despite embryo transfer. Intralipid failed to improve live delivered pregnancy rates in women with prior miscarriage or previous failure with embryo transfer [35].

Abnormal natural killer (NK) cell activity has been suggested to be a high-risk factor associated with unexplained recurrent spontaneous abortion (URSA). Intralipid, like immunoglobulin, is able to lower the activity of NK cells, which has been reported to be useful for improving URSA outcomes in pregnancy. This study aimed to determine whether intralipid could be used as an alternative treatment to intravenous immunoglobulin (IVIG) which is expensive and has many side-effects.

A prospective, randomized clinical trial was conducted from December 2010 to December 2012. Eligible participants were matched and sorted randomly into the intralipid and the IVIG group. The primary outcome was the rate of successful pregnancy. In addition, comparisons of peripheral NK cell activities were accessed by flow cytometry. Moreover, the effects of intralipid on trophoblasts were investigated using a Matrigel assay with the JEG-3 cell line.

Seventy-six patients in the intralipid group and 78 in the IVIG group completed the trial. There were no statistically significant differences in successful pregnancy rates between the two groups (92.1 vs 88.2 %, P=0.415). The reduced NK cell concentrations revealed the cytotoxic effects of the treatments in both groups. The invasive ability of JEG-3 cells was inhibited during co-culture with patient PBMCs. However, the inhibitory effect could be alleviated if the patient PBMCs were stimulated with intralipid.

Intralipid can be used as an alternative treatment to IVIG for URSA, and its potential mechanism of action may occur by regulating NK cell function and promoting trophoblast invasion [36].

The regulation of uterine and peripheral blood natural killer (NK) cells has been associated with problems related to reproductive immunology such as recurrent pregnancy loss (RPL), implantation failure or preeclampsia. NKp46, one of the natural cytotoxicity receptors (NCRs), is a unique marker that functions in NK cell cytotoxicity and cytokine production. Expression of NKp46 on NK cells is lower in women with recurrent pregnancy loss and pregnancy-induced hypertension. Moreover, expression of NKp46 on peritoneal fluid NK cells is lower in women with pelvic endometriosis. Therefore, evaluation of NKp46 on peripheral blood NK cells may provide a means of screening for reproductive abnormalities. Recently, a new type of NK cell, the NK22 cell, has been reported. This cell may be a regulator not only of the mucosal barrier but also of reproduction. For women with RPL showing abnormal uterine and/or peripheral blood NK cells, both intravenous immunoglobulin treatment and intralipid treatment have been reported. The effects of these treatments are still controversial, and further studies are needed in order to clarify their true impact [37].

Treatment of patients with recurrent pregnancy losses and recurrent implantation failure can be instituted only when the underlying etiology is determined. Embryo-secreted preimplantation factor (PIF) is essential for implantation and adequate trophoblastic invasion. Deficiency of PIFaffects the outcome of the pregnancy leading to recurrent pregnancy losses. Synthetic PIF modulates the outcome of the pregnancy decreasing the incidence of recurrent implantation failure and recurrent pregnancy losses. In this article a thorough search is done regarding the data published for diagnoses of reproductive failure and its treatment. The effect of immunoglobulin (Ig), intralipid, heparin, aspirin, progesterone, estrogen, and granulocyte colony stimulating factor (G-CSF) is taken into consideration. Heparin, aspirin, and progesterone have successfully shown to decrease the incidence of recurrent pregnancy loses; whereas G-CSF, intralipids, estrogen, and Igs have shown success in the treatment of the recurrent implantation failure and recurrent pregnancy failure. The pregnancies treated with Igs and intralipids showed equal outcome when evaluated and compared. The place of intralipid in reducing natural killer (NK) cells has been discussed [38].

Before effective treatment for reproductive failure can be instituted, the cause of the failure must be determined. A search of PubMed was made to identify the published data regarding diagnosis and treatment of reproductive failure. Results were compared with the frequency of

antiphospholipid antibodies (APA) in 2995 women with histories of unexplained infertility, recurrent implantation failure, recurrent pregnancy loss, and fertile women. In addition, pregnancy outcomes among 442 women experiencing reproductive failure and elevated NK cell activity after treatment with intravenous immunoglobulin (IVIg) (N=242) or intralipids (N=200) were compared. The prevalence of APA was the same among women with the diagnosis of unexplained infertility, recurrent implantation failure, and recurrent miscarriage. Heparin and aspirin are successful in the treatment of elevated APA among women with recurrent miscarriage but not with recurrent implantation failure. IVIg has been successful in the treatment of recurrent miscarriage and recurrent implantation failure among women with elevated APA and/or NK cell activity. When the pregnancy outcomes of women with a history of reproductive failure and elevated NK cell cytotoxicity treated with intralipid were compared with women treated with IVIg, no differences were seen. Immunotherapy for treatment of reproductive failure enhances live birth but only in those women displaying abnormal immunologic risk factors [39].

The purpose of this study was to compare the ability of intravenous immunoglobulin (IVIg), intralipid and soluble human leukocyte antigen (sHLA)-G to suppress natural killer (NK) cell cytotoxicity in an *in vitro* assay.

Blood samples taken from 275 women experiencing reproductive failure were analyzed for NK cytotoxicity and the suppression of NK cytotoxicity by IVIg 4 and 2 mg/mL (n=275), intralipid 18 and 9 mg/mL (n=275) and sHLA-G 70 and 35 ng/mL (n=50) using immunofluorescent labelled K562 cells as targets and flow cytometry.

Natural killer cytotoxicity was suppressed in all samples. Among patients with normal NK cell activity, IVIg suppressed NK cytotoxicity by 44.9 +/- 8.1%, intralipid suppressed NK killing by 45.2 +/- 8.3% and sHLA-G suppressed by 49.0 +/- 9.2%. When specimens with abnormal NK activity were observed for suppression of cytotoxicity, IVIg suppressed by 38.9 +/- 5.4%, intralipid suppressed by 39.8 +/- 6.2% and sHLA-G suppressed by 39.9 +/- 5.0%.

Intravenous immunoglobulin, intralipid and sHLA-G suppressed NK cell cytotoxicity with equal efficacy in an *in vitro* assay [40].

To elucidate the effects of parenteral nutrition on the immunocompetence, we administrated parenteral nutritional support to the malnourished gastric cancer patients during perioperative periods [41]. Changes of peripheral blood natural killer cytotoxicity (NKC) activity (LDH enzyme-release assay) and T lymphocyte subsets (OKT series monoclone antibody indirect fluorescent assay) were monitored before and after nutritional support. The results showed that one week of pre or postoperative parenteral nutrition significantly increased the NKC activity, T-helper, and T-helper/T-suppressor ratio. The total T lymphocytes count may also increase. T-suppressors remained unchanged no matter whether nutritional support was given or not. The authors believe that perioperative nutritional support could improve the immunocompetence of gastric cancer patients to some extent and promote the restoration of immune depression caused by operation, but it could not eliminate the effects of immune depressing factor produced by the tumor. Intralipid can be used properly as non-protein energy source without immuno depressing effects [41].

The effect of Intralipid on the natural killer (NK) cell activity of healthy male Fisher 344 adult rats was investigated. They were cannulated via the right jugular vein and continuously infused for five days with: normal saline plus heparin, 5% Intralipid plus heparin, or 10% Intralipid plus heparin. Control groups comprised of cannulated rats receiving no infusion and rats undergoing no operative procedures. Following the five-day infusion, rats were exsanguinated under ether anesthesia and mononuclear cells (MNC) harvested from the peripheral blood. NK activity was measured in a standard four-hour 51Cr release assay against YAC-1 target cells. NK cell activity in rats infused with 5% Intralipid did not differ significantly from rats in both control groups or rats infused with saline. Infusion of rats with 10% Intralipid resulted in a significant increase in NK activity compared with all other groups [42].

Natural killer cell activity and antibody-dependent cellular cytotoxicity were measured in 12 surgical patients before and after a 24-h infusion of 1 litre of 20 per cent Intralipid. A further 12 patients received an equivalent amount of physiological saline and acted as controls. Neither Intralipid nor physiological saline had any effect upon natural killer cell activity or antibody-dependent cellular cytotoxicity under the conditions of this study [43].

The effect of a continuous infusion of a soybean oil emulsion on immune function was evaluated in 40 malnourished patients who were randomized to receive preoperatively either a 25% glucose-5% amino acid solution (group G) or a 15% glucose-3.3% Intralipid-5% amino acid solution (group G-F). Average length of total parenteral nutrition (TPN) was 10.3 +/- 0.9 days for group G and 9.0 +/- 0.8 days for group G-F. Initial nutritional status and response to TPN were similar for both groups. Immune function was assessed before TPN and after nutritional repletion prior to surgery for each patient. The levels of immunoglobulins, C3, C4, circulating B lymphocytes and T lymphocytes, suppressor T lymphocytes, natural killer cell activity, and monocytes were normal before TPN and after nutritional therapy. However, the total number of T cells and helper T cells were low before TPN and remained so after TPN. In addition, lymphocyte function measured by the lymphocyte blastogenic response to phytohemagglutinin and pokeweed mitogen was depressed prior to TPN and was not improved by either regimen. Neutrophil chemotaxis and bactericidal activity were not affected by either nutritional regimen while neutrophil phagocytosis was enhanced before TPN and remained elevated throughout TPN with either regimen. There were no differences in infection rates during TPN. The addition of Intralipid to the TPN regimen did not alter immune function in these patients who showed depressed cell-mediated immunity before TPN compared with the standard glucose TPN regimen [44].

The effect of intralipid, a lipid emulsion used in total parenteral nutrition, on cellular cytotoxicity for herpes simplex virus (HSV)-infected cells was analyzed. *In vitro*, intralipid inhibited antibody-dependent cellular cytotoxicity (ADCC) of lymphocytes, monocytes-macrophages, and polymorphonuclear leukocytes and natural killer cytotoxicity of lymphocytes for radio labelled HSV-infected liver cells. This was due to an effect on the leukocytes, rather than on the target cells. Intralipid did not affect leukocyte viability but inhibited the expression of leukocyte Fc receptors necessary for cytotoxicity. *In vivo*, intralipid inhibited murine ADCC and completely nullified the protection against lethal infection with HSV in neonatal mice afforded by the administration of human leukocytes and antibody. These data suggest that high levels of circulating intralipid may interfere with antiviral immunity in humans and predispose hosts who are already compromised to severe viral infections [45].

Intralipid vs. Pre-Implantation Factor (PIF)

Despite the use of adjuvant therapies, the cumulative proportion of live births remains at ~40%. Accumulating data show that low pregnancy rates, even in the presence of high fertility rates, are due to implantation failure. The present study aimed to identify and construct a profile of proteins that react with preimplantation factor (PIF) and to provide an understanding into the molecular mechanisms by which PIF promotes trophoblast invasion. Cytoplasmic proteins were immune precipitated with biotin-labelled synthetic PIF or intralipid and scrambled PIF (PIFscr). The protein profiles were analyzed using isobaric tags for relative and absolute quantification coupled with mass spectrometry. Immunoprecipitation and western blot analyses were used to assess the interactions between PIF and myosin heavy chain 10 (MYH10) and heat shock protein family D1. Small interfering RNA-based silencing was performed to examine the function of MYH10. In the results of the present study, 21 proteins were identified with interactions with PIF. The immunoprecipitation and western blot analyses revealed an interaction between PIF and MYH10. Silencing of the expression of MYH10 in HEC-1-B cells significantly attenuated cell migration and invasion capacities. These data support the conclusion that MYH10-mediated cell migration and invasion act in conjunction with PIF to promote the trophoblast invasion procedure [46].

Treatment of patients with recurrent pregnancy losses and recurrent implantation failure can be instituted only when the underlying etiology is determined. Embryo-secreted preimplantation factor (PIF) is essential for implantation and adequate trophoblastic invasion. Deficiency of PIFaffects the outcome of the pregnancy leading to recurrent pregnancy losses. Synthetic PIF modulates the outcome of the pregnancy decreasing the incidence of recurrent implantation failure and recurrent pregnancy losses. In this article a thorough search is done regarding the data published for diagnoses of reproductive failure and its treatment. The effect of immunoglobulin (Ig), intralipid, heparin, aspirin, progesterone, estrogen, and granulocyte colony stimulating factor (G-CSF) is taken into consideration. Heparin, aspirin, and progesterone have successfully shown to decrease the incidence of recurrent pregnancy loses; whereas G-CSF, intralipids, estrogen, and Igs have shown success in the treatment of the recurrent implantation failure and recurrent pregnancy failure. The pregnancies treated with Igs and intralipids showed equal outcome when evaluated and compared. The place of intralipid in reducing natural killer (NK) cells has been discussed [47].

Embryo-secreted preimplantation factor (PIF) is necessary for, and its concentration correlates with, embryo development in humans by promoting implantation and trophoblast invasion. Synthetic PIF (sPIF) modulates systemic immunity and is effective in autoimmune disease models. sPIF binds monocytes and activated T and B cells, leading to immune tolerance without suppression. This study examined the effect of sPIF on natural killer (NK) cell cytotoxicity in 107 consecutive non-selected, nonpregnant patients with recurrent pregnancy loss (RPL) and 26 infertile IVF patients (controls). The effects of sPIF, intravenous gamma immunoglobulin (Ig), Intralipid and scrambled PIF (PIFscr; negative control) on NK cell cytotoxicity to peripheral-blood cells were compared by flow cytometry of labelled-K562 cell cytolysis. The effects of sPIF and PIFscr on whole-blood NKCD69+ expression were also compared. In patients with RPL, sPIF inhibited NK cell cytotoxicity at doses of 2.5 and 25 ng/ml (37% and 42%) compared with PIFscr (18%; $P<0.001$), regardless of the proportion of peripheral-blood NKCD56+ cells to lymphocytes. Pre-incubation of blood from infertile patients with sPIF for 24h decreased NKCD69+ expression versus incubation with PIFscr ($P<0.05$). In conclusion, sPIF inhibits NK cell cytotoxicity by reducing NKCD69 expression, suggesting a significant role in RPL patients. There is a continuous search to identify safe and effective agents to counteract recurrent pregnancy loss (RPL). Preimplantation factor (PIF) secreted by the embryo at the 2-cell stage is present throughout viable pregnancy but absent in nonviable pregnancy. Its immunomodulatory (not suppressive) effects promote embryo acceptance and maintenance by mother/host, control inflammation, facilitate uterine environment and placental embedding. Synthetic PIF (sPIF) was used to complete PIF's role as a targeted, safe treatment for immune-based RPL. Previous reports showed sPIF's significant protective systemic effect against maternal factors present in RPL serum. Herein is examined sPIF's ability to inhibit the local protective toxicity induced by natural killer (NK) immune cells in a representative number of RPL patients. When elevated in blood, NK cells are associated with RPL. Low-dose physiological sPIF was highly effective to inhibit NK cell toxicity. Side-by-side comparison showed that sPIF is equally effective at a lower dose than intravenous gamma immunoglobulin or Intralipid treatment currently used. The sPIF effect on NK cells was targeted, indicating specific action. Overall, sPIF may represent a safe, effective and nontoxic immune-based therapy against RPL [48].

Conclusion

Intralipid treatment of Preeclamsia/Exclampsia is first suggested in the medical literature.

Intralipid interacts with the natural killer cells as well as

with the mitochondria and intracellular calcium.

Animal studies should be done in order to evaluate this new treatment modality.

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